Zoran M. Pavlovic *Editor*

Glutamate and Neuropsychiatric Disorders

Current and Emerging Treatments

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ISBN 978-3-030-87479-7 ISBN 978-3-030-87480-3 (eBook)
https://doi.org/10.1007/978-3-030-87480-3

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

“Alle Ding sind Gift und nichts ohn’ Gift; allein die Dosis macht, das ein Ding kein Gift ist.”
“All things are poison, and nothing is without poison; the dosage alone makes it, so a thing is not a poison.”
—Paracelsus

“Die Dosierung macht das Gift: Von der Glutamat-vermittelten Exzitotoxizität zur Glutamat-vermittelten Neuroplastizität.”
“The Dosage Makes the Poison: from Glutamate-mediated Neurotoxicity to Glutamate-mediated Neuroplasticity.”
—Zoran M. Pavlovic

Foreword

The brain and neuropsychopathology continue to be the source of enduring mystery and limitless exploration. Through advances in technology, our quest to probe the brain's capabilities has enabled us to reduce this complex organ to a finite list of molecules and cell types. Yet, it continues to be the seat of seemingly infinite possibilities. The fact that it works well at all, let alone most of the time in most of us through its circuit redundancy and functional checkpoints, is perhaps the most unfathomable mystery that it holds. *Glutamate and Neuropsychiatric Disorders: Current and Emerging Treatments* offers a broad and deep exploration of one aspect of the cosmos that is the brain. Glutamate is the stuff of thoughts and memories, excitatory and elating. Yet, in excess, it is devastating in its effects. This simplistic view of glutamate as the primary excitatory neurotransmitter belies the constellation of interactions in which it has been implicated across wide-ranging neuropsychiatric syndromes, disorders, and symptoms. This thoughtfully curated collection catalogues a compendium of receptors, a host of mechanisms, and the complex connections glutamate co-experiences with its fellow synaptic travelers.

This book arrives in the context of a rapid expansion of our technological ability to identify targets, craft molecules, and measure human behaviors and activities with micrometer accuracy through tools powered by machine learning and artificial intelligence. With such tools available the traditional dualities of mind–body, mind–brain, and psychology–neurology now seem as ever-eclipsing Venn diagrams. Glutamate, as we know it, could arguably be the prototypical neurotransmitter that brings the disciplines of neurology and psychology to peak obscuration.

For the psychiatrist, this text offers new insights into anxiety, stress, and impulse control disorders, and new targets for mood and psychotic disorders. For neurologists herein are implications for multiple sclerosis, migraine, and the other side of the learning and memory coin: dementia. How one molecule and its contingent of transporters impact such far-reaching diagnoses and symptom domains is perhaps no mystery in the end. As with the worlds beyond our sight, our tools have just begun to uncover that which was there all along, waiting for discovery. This collection of works from these intrepid researchers reads as a discovery lab's

ambitious “to do list” and the work has only just begun. And the potential impact on human brain health—human health—from future treatments is as inspiring as the task is daunting. Space was never our final frontier. The mind is. And glutamate could be a master key to unlock many of its mysteries.

Otsuka Pharmaceutical Development & Commercialization, Princeton, NJ, USA

Julie Adams, MD, MPH

Foreword

As a clinician, my daily work is helping patients suffering from different kinds of neuropsychiatric disorders to overcome the disease and get back to their everyday lives. As we all know, the current pharmaceutical treatments for those disorders could be helpful but not good enough. For example, using antidepressants could help more than two-thirds of unipolar depression patients achieve "remission," but there are still abundant patients who could be qualified as "treatment-resistant." Which kind of treatments could better help our patients? This is a vitally important question for both clinicians and researchers.

One year ago, I suddenly received a letter from Dr. Zoran M Pavlovic from Serbia. He introduced his excellent work in that warm greeting letter, the book entitled *Modulators of Glutamatergic Signaling as Potential Treatments for Neuropsychiatric Disorders*. Upon his introduction, I realized that the book's first edition received excellent reviews by leading psychiatrists and psychopharmacologists from both academia and industry. At the end of his letter, he invited me to participate in the Book Proposal Review for the second edition.

Why me? A young Chinese psychiatrist without being directly involved in glutamatergic research? I have been thinking of this for a long time until one day, I joined a live symposium at the European Congress of Psychiatry to discuss the treatment of depression. I suddenly realized that it is not about the background or personal resume. It is about the integration of academics, just as "when east meets west." The world needs us to be more deeply involved together to overcome the great challenge in the age of brain science.

Glutamate is the most diffused amino acid in the brain throughout the human life span, which is involved widely in neuronal growth and differentiation, synaptic plasticity, learning and memory consolidation, arousal, and behavior. The current book, entitled *Glutamate and Neuropsychiatric Disorders: Current and Emerging Treatments*, contains 20 chapters written by more than 30 eminent psychiatrists and neurologists from Europe, the USA, Australia, and Southern America. In this book, readers could catch the most recent preclinical and clinical evidence about glutamate, the potential mechanism, and emerging treatments in various neuropsychiatric

disorders, including chronic stress, chronic pain, migraine, epilepsy, amyotrophic lateral sclerosis/motor neuron disease (ALS/MND), multiple sclerosis (MS), ischemic stroke (IS), Parkinson's disease (PD), Alzheimer dementia (AD), attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (ASD), schizophrenia, bipolar disorder (BD), major depressive disorder (MDD), posttraumatic stress disorder (PTSD), anxiety disorders and obsessive-compulsive disorder (OCD), and substance use disorders (SUD).

Last but not least, congratulations to all the editors on their success in assembling such academic work! It is my great pleasure to welcome this book and wish it a wide distribution and recognition.

Shanghai Mental Health Center,
Shanghai Jiao Tong University School
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Jun Chen, MD, PhD

Foreword

Regulation of glutamate reminds me of the role of a lion tamer at the circus. Too much glutamate neurotransmission can have deadly consequences to neural circuitry, and too little glutamate signaling can be profoundly dulling. Because glutamate is the single most common excitatory neurotransmitter in the human CNS, it plays a foundational role in a number of brain circuits implicated in the pathophysiology of mental and neurological disorders. Under normal circumstances, activity-dependent, bidirectional control of glutamatergic synaptic function is thought to contribute to many forms of experience-determined plasticity, including important elements of cognition such as learning and memory. Consequently, there is burgeoning interest in glutamatergic targets for the treatment of a variety of psychiatric, neuropsychiatric, and neurological syndromes. This has resulted in the commercialization of new treatments, most recently esketamine nasal spray for treatment-resistant depression in adults and for depressive symptoms in adults with major depressive disorder with acute suicidal ideation or behavior. In the case of schizophrenia, attempts are ongoing in Phase III clinical trials to address the cognitive impairment associated with this disorder by tweaking the ionotropic N-methyl-d-aspartate (NMDA) glutamate receptor. In neurology and neuropsychiatry, amantadine, an NMDA receptor inhibitor, has been noted to ameliorate both hypo- and hyperdopaminergic states.

In this volume, Dr. Pavlovic has curated an overview of these and other clinically relevant issues that will help readers contextualize the practical and research implications of glutamatergic signaling for both current treatment and future drug development. An impressive array of experts (including Dr. Pavlovic) serve up reviews about the overarching role glutamate has in the stress response: in neurological disorders such as amyotrophic lateral sclerosis, motor neuron disease, multiple sclerosis, Parkinson's disease, ischemic stroke, migraine, epilepsy, and pain management; neuropsychiatric disorders such as Alzheimer dementia; childhood-onset disorders such as attention deficit/hyperactivity disorder and autism spectrum disorder; and psychiatric illnesses such as bipolar disorder, major depressive disorder,

posttraumatic stress disorder, anxiety, obsessive-compulsive disorder, substance use disorders, and schizophrenia.

Taming glutamate requires knowledge of both metabotropic and ionotropic receptors, and not only NMDA receptors, but also the "companion" ampakine and kainate receptors that operate in tandem with NMDA receptor, and the recognition of the role of co-agonists such as glycine and D-serine. All of these can be subject to pharmacological manipulation. As you will see in the following pages, it is not only about glutamatergic neurons, but also about gamma-aminobutyric acid interneurons, astrocytes, microglia, and oligodendrocytes. How all of this can ultimately regulate the monoamines (notably dopamine, serotonin, and norepinephrine), as well as other "downstream" neurotransmitters, holds the key to a better understanding of CNS disease and potential treatments.

New York Medical College, Valhalla, NY, USA

Leslie Citrome, MD, MPH

Foreword

Dr. Pavlovic and the authors he has assembled are to be congratulated for taking on a Herculean task to produce the book, *Glutamate and Neuropsychiatric Disorders: Current and Emerging Treatments*. Glutamate richly deserves such a comprehensive treatment, and readers will have a wealth of information on this ubiquitous and complex neurotransmitter which has been implicated in so many different conditions.

To put my comments in perspective, it might be useful to give some background on me. I went to medical school to understand the relationship between the brain and human behavior and have been fortunate to spend 40 years in academic medical career doing just that particularly in the area of new psychiatric drug development. I did two fellowships in anatomical pathology with a focus on neuropathology, followed by a general medicine-psychiatry internship, and a psychiatry residency with an emphasis on neurobiology.

It is that perspective that I think this multi-authored text under the guidance of Dr. Pavlovic will be an aid to healthcare professionals from many different specialties as well as basic researchers and student interested in understanding how one transmitter may play a role in such otherwise disparate conditions from autism spectrum disorder to Alzheimer's disease—the former presenting early in life and the latter relatively late in life.

The book covers a wide range of disorders some of which might be considered more neurological, others more psychiatric, and still others more general medical. It is to Dr. Pavlovic to have been able to bring so many authors together to produce such an encyclopedic text.

In thinking about this foreword, I reviewed the topics and regrouped them into the following table to illustrate the breadth of the book. The classic neuropathology refers to conditions in which gross or histopathological or other laboratory findings (e.g., electroencephalography) have established. Nonclassical neuropathy refers to conditions where such findings are not as well established, but instead more biochemical or functional features are either established or proposed.

This book explains how specific dysregulation of the major excitatory neurotransmitter in the brain, glutamate, could play a role in the pathophysiology of all

these conditions and hence why it can be a focus for effective therapeutic intervention in these otherwise disparate illnesses.

It will be a valuable resource for all of those interested in understanding this neurotransmitter and the pathobiologies underlying these illnesses.

Classic Neuropathology
Ischemic Stroke Andrés Da Silva Candal
Alzheimer's Dementia Markku Kurkinen
Amyotrophic Lateral Sclerosis (ALS) Andrea Diana
Multiple Sclerosis Anna Pittaluga
Parkinson's Disease Fabrizio Gardoni
Epilepsy lberto E. Musto
Nonclassical Neuropathology
Listed usually under Neurology
Migraine Anna Andreou
Listed usually under Psychiatry
Bipolar Disorder Kostas N. Fountoulakis
Schizophrenia Luis F. Callado
Posttraumatic Stress Disorder (PTSD) Maurizio Popoli
Impulsive Aggression Alan R. Felthous
Substance Use Disorders (SUD) Richard L. Bell
Anxiety Disorders Zuleide M. Ignacio
Listed under both
Chronic Stress Zoran M Pavlovic
Autism Spectrum Disorder (ASD) Carla Sogos
Attention Deficit Hyperactivity Disorder (ADHD) Greg A. Gerhardt
Listed usually under General Medical
Chronic Pain Kathleen Holton

University of Kansas School of Medicine, Wichita, KS, USA
27 May 2021

Sheldon Preskorn, MD

Preface

My "Glutamate Journey"

Introduction

Reflecting on my 25-year long, as I call it, "glutamate-based" career, even today, I vividly remember the lovely sunny day on May 5, 1994, when my father Milan taught me how to tie a tie and get ready for my first day of work at ICN Yugoslavia. The pharmaceutical company Galenika located in Zemun, in the vicinity of Belgrade, had been recently bought by a California-based ICN Pharmaceuticals founded and chaired by a Serbian-born American businessman Mr. Milan Panic, a biochemist and a former Yugoslavian national champion in cycling. The first meeting was with my line manager, who told me that my "startup" assignment would be to intensify a promotional campaign for the first-ever CNS blockbuster Prozac (Fluoxetine), which was recently in-licensed from Eli Lilly. Due to the initial reluctance of Serbian psychiatrists to administer an SSRI antidepressant for the first time, especially to their hospitalized patients with severe depression, I decided, together with two key opinion leaders, Professor Ivana Timotijevic, MD, and Professor Vladimir R Paunovic, MD, to conduct a small open-label clinical study, to compare antidepressant effects of fluoxetine, between outpatients and inpatients, with a clinical diagnosis of major depressive disorder (MDD). The study was a major success, as it showed beneficial antidepressant effects of Flunirin (fluoxetine's brand name in Serbia) on both patient populations, and in addition, in comparison to the tricyclic and tetracyclic antidepressants (standards of care at that time), had fewer adverse events and more rapid onset of action. As I thought that study results might be interesting to present at an international conference, I compiled the essential data from the clinical study report and created my first poster, with study data published in the Abstract Book of the International College of Neuropsychopharmacology (CINP) Congress that took place in Melbourne, Australia, in 1995. A year later, Flunirin was the most prescribed antidepressant in Serbia and Montenegro. My next assignment was to coordinate a neurology project together with my colleagues from

F. Hoffmann-La Roche, related to the introduction of a sustained-release version of levodopa benserazide combination drug Madopar HBS, which just got EU approval for the treatment of night-time problems in patients with Parkinson's disease (PD), to the Serbian market. A key opinion leader for PD, Associate Professor Vladimir S Kostic, MD, Chief of Department for Movement Disorders at the Institute of Neurology in Belgrade, was at that time attending his advanced training at Professor Serge Przedborski MD's Lab at the Columbia University Motor Neuron Center in New York, who pioneered the investigation of molecular mechanisms of neuronal death in the MPTP model of PD. Our daily phone conversations usually started at 5 AM ECT, 11 AM Belgrade time, when we discussed various aspects of PD night-time symptoms, both disease and treatment related. These 15- to 30-minute consultations were extremely helpful for boosting my expertise in PD necessary for upcoming promotional activities, including setting up a local post-marketing clinical study.

Booklet on glutamate role and Lamotrigine treatment of seizures in Rett syndrome, 3rd European Congress of Epileptology—*Poland (1998)*

In August 1996, I got a fantastic opportunity to move from a mid-size to a top-five big pharma company, GlaxoWellcome (GW) plc, now GSK, as a Medical and Regulatory Officer at their Representative Office in Belgrade. That was the time and place where my "romance" with glutamate and glutamatergic treatments began. It turned out that my first "GW baby" was lamotrigine, a phenyltriazine synthesized at Wellcome Laboratories in the UK in the early 1980s in response to an unmet need for an antiepileptic drug with an improved safety profile and broader therapeutic efficacy. I still recall that I was puzzled by its unique mechanism of action, as it was the first anticonvulsant demonstrating effects on glutamate neurotransmission. At that time, Lamictal (lamotrigine's brand name) was solely indicated for patients with treatment-resistant epilepsy (TRE). A couple of weeks after I joined GW, word of mouth led to one of the most prominent child epileptologist, Associate Professor Nebojsa Jovic, MD, inviting me to his office to supply him with the latest scientific publications on lamotrigine because he was very keen to begin treating his pediatric and adolescent patients with refractory seizures with lamotrigine. He was especially interested in administering Lamictal to several of his young female patients with Rett syndrome (RS) and poorly controlled seizures on standard antiepileptic drugs, as it was observed that children with RS exhibited high levels of glutamate in the cerebrospinal fluid (CSF). At that time, there was only one case report published by Uldall et al. in *Neuropediatrics* journal from 1993 about the use of lamotrigine in this rare and severe neurodevelopment disease, so I strongly suggested to Professor Jovic to closely monitor the safety of his patients as several cases of lamotrigine-induced Steven-Johnson syndrome were already reported in adults with TRE. We also discussed which instruments would be most appropriate for capturing treatment effects in addition to the EEG analysis. Once the clinical trial was completed, I submitted a study report to my colleagues at GW headquarters in Greenford, UK. They immediately proposed to publish the study results as a booklet and distribute it to the epileptologists during ILAE (International League against Epilepsy), 3rd European Congress of Epileptology in Warsaw, Poland, in 1998. It was a

brilliant idea, as the booklet was "sold out" during the first day of the conference. On top of that, after the meeting, other GW offices worldwide asked for my permission to use it when locally promoting Lamictal for TRE.

Poster presentation on Lamotrigine use in a treatment-resistant patient with bipolar disorder at XXIst CINP Congress—*Scotland (1998)*

At that time, I did not know that my love at first sight with lamotrigine would end up in a long-term relationship with total commitment from both sides. Still, the next *Rendez-Vous* came just after the conference in Warsaw, as GW initiated a Phase III study on lamotrigine use in bipolar disorder (BD). At that time, GW had limited experience with clinical trials in psychiatry, so they looked for the CRAs with psychiatric expertise and recruited several prominent psychiatrists in the UK and the USA to coordinate a very ambitious clinical development program. The trials included patients with bipolar depression, bipolar mania, and the "rapid cycling" variant of BD. Although I was thrilled to be a part of the most extensive global placebo-controlled studies ever done in BD, I was particularly excited to be involved in a rapid cycling study, as it was one of the first (if not "the" first) FDA- and EMA-approved psychiatric study, which used the electronic case report forms (eCRFs), which allowed me to monitor patient data via an Internet cable connection, by using my laptop at the office, without the need to visit Professor Miroslava Jasovic-Gasic (MD) in person. As a final-year resident in psychiatry, I also spent a certain amount of time at the Institute of Mental Health. One day I examined and took anamnesis of a patient with BD who was intolerant to side effects of lithium, experiencing a full-blown depressive episode. I consulted with my senior colleague Professor Timotijevic on how to proceed with a treatment-resistant patient, and we agreed that the best solution would be to add lamotrigine to his ongoing antidepressant regimen. Surprisingly for both of us, adding lamotrigine reduced his depressive symptoms after only 2–3 weeks of treatment. Excited by the rapid and clinically significant effects of lamotrigine, and with great enthusiasm, I reached out to my GW colleague based in Research Triangle Park in North Carolina, in the USA, Gary Evoniuk, PhD, a Director of Medical Communications at that time, who helped me in transforming raw patient data into succinct and visually attractive poster presentation for the upcoming CINP Meeting in Glasgow. I was very flattered when the external coordinator for the Lamictal in Bipolar Disorder Study Project, Professor Joseph R Calabrese, MD, from Case Western Reserve University School of Medicine, came to me during the poster session and praised the way we managed a difficult-to-treat bipolar patient. Interestingly, this was the first European case report ever published, just a year after Professor Gary Sachs, MD, at the Department of Psychiatry, Massachusetts General Hospital in Boston, reported about positive results of using lamotrigine in his case series study with treatment-resistant bipolar patients.

Publication of case reports of my patients with impulsive aggression and alcohol use disorder treated with Lamotrigine—*Serbia (2008–2010)*

Once I completed my residency and got my board certification in psychiatry in 2002, I started my private practice a couple of months later. My first patient was diagnosed with the most severe disorganized, difficult-to-treat type of schizophrenia,

refusing to take medications due to a high level of extrapyramidal symptoms (EPS) he was experiencing while on haloperidol. His parents were also very concerned about his physical aggression bursts, mainly toward those closest to him. I decided to switch him to a second-generation antipsychotic risperidone at maximum dosage, which immediately reduced his EPS symptoms. In contrast, I noted no improvements in symptoms related to his impulsive aggression. My next step was to add clozapine to his ongoing antipsychotic regimen. Still, I did not observe any significant changes in his aggression levels measured by the Overt Aggression Scale. Coming back from one of my house calls, I suddenly remembered that one of my colleagues administered valproate to his autoaggressive patient with borderline personality disorder (BPD). When I came home, I immediately started browsing PubMed. I noticed the article by Professor Eric Hollander, MD, reporting that divalproex was superior to placebo in reducing impulsive aggression in patients with borderline personality disorder (BPD). According to him, pretreatment trait impulsivity symptoms and state aggression symptoms predicted a favorable response to divalproex relative to placebo. I also found two clinical studies demonstrating the anti-aggressive properties of lamotrigine. The first one by Beran and Gibson, with intellectually challenged patients, was published in 1998, and the second by Tritt et al. from 2005, showing that lamotrigine reduced aggression in female patients with BPD. Moreover, I found several preclinical animal studies conducted with other compounds with glutamatergic properties. One of them was from 1982 by Miczek and Tyler about the effects of phencyclidine on aggressive behavior in mice, the other one by Takahashi reporting on the effects of ketamine in animal models of aggression from 1984, and the most recent one from 1992 by Lu et al. regarding the role of glutamate NMDA receptors in aggression. This compilation of evidence-based data encouraged me to start with lamotrigine treatment, and once my patient was maintained on a dosage of 200 mg/day for several weeks, I observed his aggression begin to diminish. This case report was published as the Letter to the Editor in the *Journal of Clinical Psychopharmacology*, which ranked as a top 5 psychopharmacology journal with an impact factor of almost 6.0 in 2008 (for comparison purposes, a psychopharmacology journal with the highest impact factor in 2019 was *Neuropsychopharmacology*, the official journal of the American College of Neuropsychopharmacology (ACNP) with an impact factor of 6.7). It was the first article that appeared in a medical journal reporting about the anti-aggressive properties of lamotrigine in a patient with a most severe type of schizophrenia. Moreover, Professor Leslie Citrome (MD) and others quoted my findings in their chapter "Understanding and Managing Violence in Schizophrenia" published in the Second Edition of the book *Comprehensive Care of Schizophrenia: A Textbook of Clinical Management*, Edited by Professor Jeffrey A. Lieberman, MD, and Professor Robin M. Murray, MD. Going to a private psychiatric practice in case you suffer from a substance use disorder (SUD) in Belgrade is much more comfortable and less stigmatized, and according to my patients, has additional benefits, as I was spending more time with them, allowing me to do motivational interviewing, and the simple

cognitive-behavioral interventions during the session. I still remember one of my older patients, with chronic drinking problems and heavy binge-drinking episodes, who did not want to go to the hospital for detoxification while being desperate due to her inability to cope with alcohol craving, which was incredibly intense for her in the morning before going to work. Although I did not have too much experience with SUD patients, I decided to accept the challenge, especially as she was my first client with alcohol use issues. I was pretty much disappointed when I found out that my colleagues were mainly prescribing Antabuse (disulfiram), a drug still used as aversion therapy in alcoholic patients. Although it was a drug with a fascinating history, discovered in 1881, and primarily used to accelerate the manufacturing process of rubber, and the first drug for the treatment of alcohol use disorder (AUD) approved by the Food and Drug Administration (FDA) in 1949, I was very skeptical about its use in my patient, due to hepatotoxicity, neurological side effects, and lack of consistent information about its optimal dosage. While searching the literature for more information about novel compounds for this indication, I stumbled upon topiramate, an antiepileptic drug with GABA-ergic and glutamatergic properties, tested in several placebo-controlled clinical trials coordinated by Professor Bankole Johnson, MD, from the University of Maryland School of Medicine. The results from these trials, published in 2004 and 2007, reported clinically significant improvements in patients' quality of life receiving topiramate. Moreover, treatment responders experienced reductions in the severity of addiction symptoms and frequency of heavy drinking episodes. The investigators also noted better overall psychosocial functioning in topiramate patients, mainly due to reduced binge drinking. However, both studies showed several limitations. Firstly, their aim was not to measure the intensity of withdrawal symptoms. Secondly, the trials' duration was only 14 weeks; therefore, they were not designed to capture topiramate's effects in preventing future relapses. This initial information about the successful use of an antiepileptic with glutamatergic properties in patients with alcohol dependence reminded me of another well-known drug used in AUD, acamprosate, a weak NMDA antagonist, which also interacts with a group one (I) metabotropic glutamate receptor type 5 (mGluR5). This drug was tested for the first time in alcohol-dependent patients in 1984, in a study by Hillemand et al. and then a year later in similar research by Lhuintre. Both investigators were impressed about its unique anti-craving properties, manifested as a decrease in "liking, wanting, urges, desires, need, intention or compulsive drinking, which are usually present during ethanol withdrawal in alcoholics." These unique findings led them to suggest that acamprosate might be considered in preventing relapse in patients with chronic drinking problems. Another study, this time in animals, by Olive and al. published in 2002, reported a reduction of the rewarding effects of alcohol with acamprosate, possibly by attenuating the ethanol-induced increase in dopamine levels in the nucleus accumbens (NAc) of rats. Unfortunately, my enthusiasm for prescribing acamprosate to my patient waned quickly, as it turned out that the drug was not

available in Serbia at that time. Therefore, I moved on with my literature search and found a paper by Rubio et al. from 2006, who noted that lamotrigine might be a treatment option for BD comorbid with AUD. Just a year later, in an animal study, Vangeliene et al. concluded the following: "The ability of Lamotrigine to reduce alcohol-seeking as well as relapse-like drinking behavior provides further support for the proposed involvement of glutamatergic and dopaminergic systems in alcohol craving and relapse, hence suggesting a good rationale for pharmacological intervention that may reduce craving and relapse in alcohol-dependent patients." Another seminal article on lamotrigine use in AUD was written by Krupitsky, who compared the effects of glutamatergic treatments (lamotrigine, topiramate, and memantine) to diazepam, and placebo, in ethanol detoxification of patients with AUD. He reported the positive effects of lamotrigine similar to those observed with traditional benzodiazepines but with fewer side effects. The authors also noted superior effects of lamotrigine over memantine and topiramate in reducing observer-rated and self-rated alcohol withdrawal severity. Moreover, they suggested that the observed between-treatment difference might reflect an advantage of lamotrigine which mainly inhibits glutamate release, over pharmacologic strategies that target individual glutamate receptors, like topiramate and memantine. Boosted by these initial findings of successful use of lamotrigine in AUD, I decided to give it a try; however, instead of administering a dose of 100 mg/day of lamotrigine like Krupitsky, I aimed for a maximum recommended dosage of lamotrigine when given as monotherapy to patients with TRE which was 200 mg/day. Another significant difference between my approach and the one of Krupitsky was that he tested the effects of lamotrigine monotherapy on alcohol withdrawal symptoms, while I added lamotrigine to a standard detoxification protocol, up-titrating it to the optimal dose of 200 mg/day, and then slowly tapered down and eventually discontinued "Benzos." This method proved to be an excellent strategy, as there was no increase in withdrawal symptoms while switching from benzodiazepine to lamotrigine. In contrast, I was hesitant about continuing the administration of lamotrigine to my patient as there was not enough information supporting long-term treatment of AUD with antiepileptic drugs. Still, I decided to follow the recommendation of Professor Johnson that people who are alcohol dependent "require continuous, and possibly lifetime pharmacological treatment, in the same manner as the provision of insulin to a diabetic is essential." Moreover, as she was my first patient using lamotrigine for relapse prevention, I kept in touch with her by phone and was very satisfied as she reported total abstinence from alcohol for the following 3 years. I described this patient in a case report published in the *Journal of Neuropsychiatry and Clinical Neurosciences*, the official journal of the American Neuropsychiatric Association, postulating that specific advantages of lamotrigine in comparison to other similar drugs might stem not only from its marked anti-craving properties but also due to its effects on decreasing depressive symptoms, often occurring during alcohol withdrawal, and usually serving as relapse triggers.

History of glutamate discovery, a mini-review on glutamate and glutamatergic drugs, and medical monitoring of the MDD study with Declogurant—*Germany (2011–2012)*

After a decade of working in my private practice, I got a job offer to relocate to Germany, the country where glutamic acid was discovered by the German chemist Karl Heinrich Ritthausen in 1866, who, while working with wheat proteins, isolated it by treating wheat gluten with sulfuric acid. While preparing a preface for my first book, I learned that even sponges that do not have a nervous system use glutamate for cell-to-cell signaling. Moreover, they too possess metabotropic glutamate receptors, so that the application of glutamate to a sponge can trigger a whole-body response that sponges use to rid themselves of contaminants. The excitatory effects of glutamate on neuronal cells were discovered by Japanese scientist T. Hayashi in 1952, who found that injections of glutamate into the cerebral ventricles of dogs could cause seizures; however, he firmly rebuffed any notions that glutamate might be considered an "excitatory transmitter." By the end of the 1950s, there was a consensus that glutamate strongly excites many central neurons but was unlikely to be a synaptic transmitter. One of the most common reasons for skepticism was the universality of glutamate's excitatory effects in the CNS, which seemed inconsistent with the specificity expected of a neurotransmitter. The first scientist who identified glutamate as a primary excitatory neurotransmitter was a neurophysiologist Professor Kresimir Krnjevic, PhD, born in 1927 in Zagreb, at that time, the Kingdom of Serbs, Croats, and Slovenes, who formally retired from McGill University in 1999 as a Professor of Physiology and died in Montreal, Canada, in 2021. While working at today's Babraham Institute, in Babraham, Cambridge, in the UK, during the early 1960s, in one of his iontophoretic experiments looking for transmitter-like actions of various substances, he noted that L-glutamate intensely excited virtually all neurons in the cerebral (and cerebellar) cortex of anesthetized cats. He informed the UK Physiological Society about his finding in September 1961. At that time, Professor Krnjevic probably did not recall Paracelsus' quote that it is the dose that only differentiates the poison from the drug. Later he briefly mentioned in one of his interviews that one aspect of glutamate's action, which later proved to be of great clinical importance, completely eluded him: its "excitotoxic" effect. Indeed, according to Professor Krnjevic, glutamate lacked any harmful effect: neurons could be excited either repeatedly or continually for many minutes without causing any apparent harm, which seemed in keeping with its proposed role as a physiological transmitter. He also remembered that he was stunned when in 1969, Professor John Olney, MD, a psychiatrist who coined the term "excitotoxicity," published a paper in the journal *Science* demonstrating that the ingestion of monosodium glutamate (MSG) caused neuronal degeneration. Fortunately, a series of discoveries during the 1970s resolved most of these doubts, and by 1980, the compelling nature of the evidence related to glutamate function as an excitatory neurotransmitter was almost universally recognized. The next stop on my *"glutamate journey"* was the

University City of Mannheim, the second-largest town in the German state of Baden-Württemberg, with approximately 300,000 inhabitants, located around eighty kilometers from Frankfurt. My first assignment in a global Contract Research Organization PRA Health Sciences was to support my colleagues from F. Hoffmann-La Roche conducting a Phase II randomized trial with a mGluR2/3 negative allosteric modulator RO4995819 (Declogurant) as adjunctive therapy in patients with MDD having inadequate response to ongoing antidepressant treatment. This engagement was an incentive for me to write a mini-review article titled *Glutamate: leader in contemporary translational neuropsychopharmacology research* for *Translational Biomedicine*, an open-access journal.

Lecture on glutamate, cocaine use disorder, lamotrigine and session co-chairing at the 2nd International Conference and Exhibition on Addiction Research & Therapy

Las Vegas, USA (2013)

Once I got encouraging results on using lamotrigine in AUD, I decided to ask my 22-year-old patient with a cocaine use disorder (CUD) who suffered from several relapses and was on the verge of dropping out from the university whether he would be interested in using it. Once his parents also gave consent, I prescribed him lamotrigine. The rationale behind my decision was not just because of my positive experiences with lamotrigine in treating patients with AUD, as I also consulted various scientific literature sources before making the final call. To my surprise, one of the first articles about the role of glutamate neurotransmission in CUD was published by Professor Peter W Kalivas (PhD) and others in 1999. In one of his subsequent articles from 2003 titled: *"Prefrontal Glutamate Release into Nucleus Accumbens Mediates Cocaine Induced Reinstatement of Drug-Seeking Behavior,"* he concluded that "pharmacological modulation of glutamate release may prove an effective target for selectively treating craving for drugs of abuse." This hypothesis was later confirmed by Schmidt and others in 2010, saying that glutamate release in the NAc of cocaine-experienced rats promoted reinstatement of cocaine-seeking behavior. After treating several of my SUD patients with lamotrigine, I concluded that lessening of subsyndromal depressive symptoms, which are frequently present in individuals with CUD, further contributed to the overall beneficial effects of lamotrigine in reducing drug-seeking, cue-induced reinstatement of addictive behaviors, and preventing relapses. The case report about the use of lamotrigine in my patient with CUD was published in 2011, and a year later, in a comprehensive review, Olive and Kalivas quoted both of my articles on SUD and suggested that based on the currently available findings, lamotrigine may be of clinical benefit in the treatment of addiction in AUD and CUD. In 2013, I was invited to an International Conference on Addiction in Las Vegas, Nevada, to speak about glutamate and glutamatergic treatment role in patients with CUD, which gave me the opportunity to meet in person with Professor Foster M Olive, PhD, from Arizona State University and ask him to present his work on the role of metabotropic glutamate receptors in SUD in my forthcoming book *Modulators of Glutamate Signaling as Potential Treatments of Neuropsychiatric Disorders*, which he gladly accepted. Another insight about lamotrigine's potential MOA in treating CUD came up while I was

preparing the preface for this book, as I found the interview that Professor Kalivas gave to the *Science Daily* in August 2018 about his preclinical study on the role of BDNF in preventing cocaine relapse in rats, saying: "An important aspect of this study is that while others have shown that BDNF is important for establishing the state of addiction, we found that it can also be used to reverse addiction." He then added, "This exemplifies that the primary effect of BDNF is to promote changes in the brain and that this capacity to change the brain contributes to how people get addicted, but also can be harnessed to remove brain pathologies such as drug addiction." Moreover, Li et al. reported about the interplay between lamotrigine and BDNF, suggesting that BDNF signaling mediated antidepressant properties of lamotrigine and that chronic lamotrigine treatment upregulated frontal and hippocampal BDNF levels and restored stress-induced downregulation of BDNF in animal experiments. Another compelling piece of evidence observed in the same study was that pharmacological inhibition of BDNF signaling completely abolished the antidepressant effect of lamotrigine. These recent developments might further contribute to the origins of lamotrigine's superiority over other glutamatergic modalities tested for SUDs.


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at the "2nd International Conference and Exhibition on Addiction Research & Therapy" during July 22-24, 2013 in Embassy Suites Las Vegas, USA.

Gedela S Babu
OMICS Group Inc., USA

Certificate for Co-chairing the session on addiction during the conference in Las Vagas (2013)

Lecture on glutamate and role of glutamatergic drugs in treating impulsive aggression, at the Department of Psychiatry, Tianjin Medical University—*China (2013)*

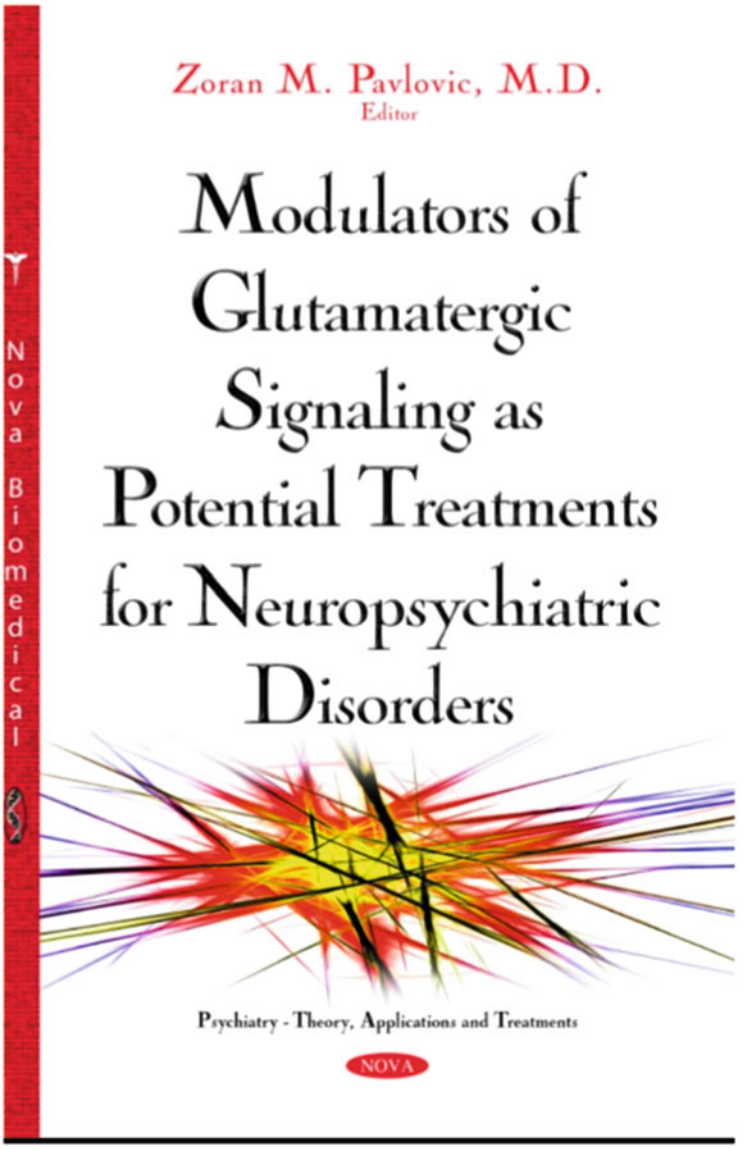
In February 2013, through one of my LinkedIn contacts, I got in touch with Professor Jie Li, MD, a psychiatrist working at Tianjin University of Medicine Mental Health Centre, who kindly invited me to give a lecture on glutamate's role and neuropsychopharmacological treatment of impulsive aggression, which coincided with the publication of a review article on a similar topic in the *Journal of Clinical Psychopharmacology*, which I wrote together with Professor Gabriella Gobbi, MD, and her lab team members Drs. Stefano Comai and Michael Tau, at McGill University in Montreal, Canada. Interestingly, McGill University was also the institution where Professor Krnjevic, who established the glutamate role as a neurotransmitter, spent most of his scientific career. Once I arrived at Beijing airport, I was greeted by Professor Li's students, and since then, until I embarked on my return flight to Frankfurt, I have experienced generosity and kindness from another dimension. Professor Li and her three students helped me learn as much as possible about China, its history, and its citizens' everyday lives during my short stay. Being a big fan of Chinese food and glutamate as a neurotransmitter, I was always intrigued about the story *of "Chinese Restaurant Syndrome,"* which goes back to a discovery of fifth taste by Dr. Kikunae Ikeda (1864–1936), a Japanese chemist and Tokyo Imperial University Professor of Chemistry who, in 1908, uncovered the chemical basis of a taste he named "umami" meaning "essence of deliciousness" in Japanese, and is considered as fifth taste or savoriness, along with sweet, bitter, sour, and salty basic tastes. The story says that in 1907, while he was eating dinner with his family, Professor Ikeda noticed that his soup was more delicious than usual; after stirring a few times, he realized the difference was the umami flavor from kombu, a species of brown macroalgae.

From that day on, Professor Ikeda continued studying the chemical composition of kelp and eventually isolated brown crystals of glutamic acid (glutamate). Dr. Ikeda then partnered with the businessman Saburosuke Suzuki and in 1908 established a company, Ajinomoto Co., Inc., developing a process for MSG's mass production by 1909. Professor Ikeda also studied other foods to see if they contained umami and confirmed that glutamate was responsible for part of the flavor of meat, seaweed, and tomatoes. He believed that humans developed a taste for glutamate because it signaled the presence of proteins. Soon, umami became a key element in culinary arts, as the Chefs realized that it kept the tastiness of dishes even though salt concentration and meal fat content was reduced. Scientists have found that humans have specific taste receptor cells for umami bundled in clusters called taste buds in the oral cavity, containing T1R1–T1R3 G-protein-coupled receptors. Interestingly, T1R binds free glutamate when food enters the mouth eliciting the umami taste that is very much appreciated in savory dishes.

Photo from the Dean's office at Tianjin Medical University, Tianjin, China (2013)

FIRST BOOK: "*Modulators of Glutamatergic Signaling as Potential Treatments for Neuropsychiatric Disorders" Heidelberg, Germany (2015)*

When I started to work from my home office in Mannheim for Worldwide Clinical Trials, as a Manager in the Psychometric Assessment Department, responsible for overseeing European Psychiatry and Neurology projects, I noticed that without the need for commuting to the office, I had a couple more hours during the day, so I decided to embark on the project related to my first book on glutamate and glutamatergic treatments. I still remember a colleague from the US Headquarters in Philadelphia, who was the first to know about my plan, immediately expressing his sincere concern by saying: "Man, you are going to get burnout syndrome." Although it was not easy to coordinate contributors' efforts dispersed in different time zones, the book was finalized in June of 2015, during my stay in Heidelberg. It contained seven chapters, written by authors from Canada, Australia, the USA, Belgium, and Serbia. Moreover, it was the first book ever published on glutamate and glutamatergic treatments, which contained the most current information about major psychiatric and neurological diseases in one place. The book received excellent reviews from world-renowned academics and industry colleagues.



Front cover of the Book "Modulators of Glutamatergic Signaling as Potential Treatments for Neuropsychiatric Disorders" (2015)

What Inspired Me to Write This Book?

My motivation to write this and the previous book stems from several personal and professional reasons.

Why Psychopharmacology and Glutamate?

My mother, Nadezda, was a big proponent of my decision to specialize in psychiatry. In the same way, my father, Milan, a pharmacist, who worked his whole life in the Serbian pharmaceutical company Galenika, supported my decision to start my career in pharmaceuticals. I sincerely enjoyed my work in the pharma industry as a pharmaceutical physician and quickly became more and more fascinated with neuropsychopharmacology. Unfortunately, my honeymoon with the CNS medications was over as soon as I stepped into my private psychiatric practice, as I was immediately confronted with the occurrence of adverse events and lack of response to available psychotropic drugs in some of my patients. These firsthand experiences facilitated my decision to administer glutamatergic treatments that were not yet tested in double-blind placebo-controlled clinical trials to my difficult-to-treat patients. Therefore, both manuscripts are also a way of expressing my sincere gratitude to patients and their families for trusting and supporting me throughout my "glutamate" journey.

Finally, I am optimistic that the book will foster future drug development initiatives, leading to the discovery of novel efficacious and safe glutamatergic treatments that will enable people with significant mental health and neurological disabilities to lead meaningful, decent, and happy lives.

Why the Book on Psychiatric and Neurological Disorders?

Personal reasons probably relate to my early and late-life experiences with my closest family members as my grandfather suffered from MDD, and my grandmother died from AD. Moreover, although trained in psychiatry, from the beginning of my career, I was always involved in parallel in neurology projects on Alzheimer's and frontotemporal dementia, PD, restless legs syndrome, primary headaches, and chronic pain. Furthermore, the first glutamatergic drug that I "got acquainted with" was lamotrigine in 1998, which was then solely administered to neurological patients with treatment-resistant epilepsy and by coincidence tested at the same time for a psychiatric indication, namely BD.

Why Is This Book Important, and Why Is It Unusual?

This book provides scientists and healthcare professionals with up-to-date and comprehensive yet practical information on glutamate-related neurobiology of major neuropsychiatric disorders and glutamatergic drugs by mixing contemporary scientific streams and clinical practice in a balanced and forward-looking way. The manuscript is also unique as it contains crucial information about glutamate neurotransmission and glutamate-based modalities for the most prevalent psychiatric and neurological disorders in one place. Authors have used their expertise to identify the most current seminal publications enriching them with their own preclinical and

clinical insights when discussing their chapter topics. Each chapter presents a concise overview of the most critical aspects of an illness and its treatment, with an additional emphasis on giving practical tools to prospective readers, so they could easily comprehend and quickly learn complex neuroscientific principles and treatment strategies. Written by more than 30 key opinion leaders from Italy, Spain, UK, the USA, Brazil, Australia, and Serbia, this book aims to bring professional and cultural diversity, hopefully contributing to the manuscript's originality.

What Is New to This Edition?

My first book on glutamate and glutamatergic treatments was published in 2015. It has seven chapters and around two hundred pages. The one that you are currently holding in your hands or browsing as the ebook on your laptop or smartphone has 20 chapters and has been streamlined to keep pace with significant developments that have taken place in the fields of neuroscience, clinical psychiatry, and neurology related to glutamate and glutamatergic treatment in the last 5 years. Other improvements include adding the *Suggested Reading* section, which provides high-level resources not cited in the chapter of vital importance for the topic discussed. Moreover, the visual aspect and concept of the book dramatically changed, with the inclusion of the numerous color and black and white *figures* (some of them were created explicitly for this book edition and published for the first time) to reinforce and visually present the main concepts learned from the written text.

Which Audience Would Benefit the Most from This Book?

The primary beneficiaries would be neurology and psychiatry specialists and residents, neuroscientists, neuropharmacologists, pharmaceutical industry, and clinical research organization professionals, academicians, students, and clinicians working with psychiatric and neurological patients with comorbidities such as cardiologists, pulmonologists, and endocrinologists. This book will also appeal to psychiatry and neurology subspecialists and clinicians working in neuroscience labs seeking an easy-to-understand yet comprehensive overview of contemporary evidence-based clinical insights backed by basic science (preclinical) research evidence.

Major Developments Related to Glutamate and Glutamatergic Treatments Since the First Book Is Published

(2015–2021)

Below is the list of main scientific, clinical, and drug development breakthroughs that occurred after the publication of my first book in 2015 till today:

- More profound understanding of the *glutamatergic system and neuroinflammation interplay in the etiopathogenesis of MDD and neurodegenerative disorders such as AD and PD*
- Comprehensive identification of *downstream pathways of glutamate-related neuroplasticity*
- *Initial evidence* about the role of glutamatergic neurotransmission in the mediation of beneficial effects of *sitting meditation, mindful movement, and yoga practices* for treating and preventing various psychiatric and neurological disorders
- Approval of the first rapid-acting antidepressant (RAAD) with a novel mechanism of action, *ketamine,* for patients with treatment-resistant MDD

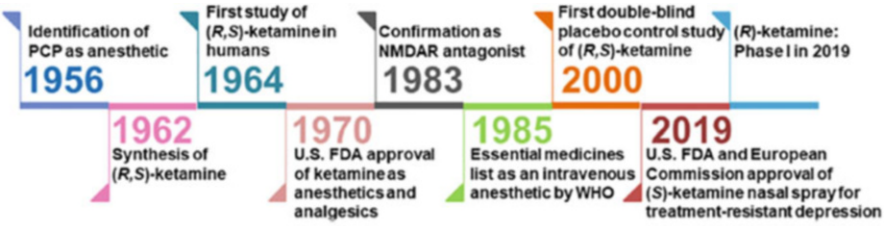


Fig. 2. Brief history of (*R,S*)-ketamine and two enantiomers from anesthetic to antidepressant.

- First clinical findings regarding the role of *glutamatergic neurotransmission in mediating treatment effects of psychedelics* like psilocybin and MDMA
- Increased knowledge about the *Neurobiology of Consciousness,* including the role of glutamatergic neurotransmission in maintaining a "cellular" consciousness
- Discovery of glutamatergic neurotransmission mechanisms underlying the development of the *newly introduced behavioral addictions* such as Internet addiction and problematic smartphone use

Five-Year Prediction About Future Directions in the Field of Glutamate and Glutamatergic Treatments

(2021–2026)

Below is my list of major scientific and pharmaceutical developments related to glutamatergic neurotransmission and glutamate-based therapeutics that I predict to occur in the next 5 years:

- Significant advancements in the *early detection of abnormalities in glutamatergic neurotransmission* enabling healthcare professionals to diagnose psychiatric and neurological diseases during the asymptomatic stage and *initiate treatment as early as possible*

- *Increased pharma and biotech investments* in discovery and development of *prophylactic glutamatergic modalities* to prevent major psychiatric and neurological disorders such as MDD and AD
- Introduction of *gene therapies for rare neurological disorders* with abnormalities in glutamate neurotransmission such as Machado–Joseph disease and Rett syndrome
- Complete *identification of glutamate-related biomarkers* and complex biological signatures for *major neuropsychiatric disorders*
- *Improvement of crisis management and intervention practices* due to availability of fast-acting ketamine-like glutamatergic drugs that will *prevent the occurrence of PTSD, anxiety, and depression* in healthy individuals affected by natural catastrophes or pandemics
- Increased investigation and knowledge about the *role of glutamate in the neurobiology of loneliness (social isolation) and social distancing*
- More intensive research on *glutamatergic biomarkers of resilience, mental toughness, hardiness, and stress susceptibility*
- *Proactive use of glutamatergic resilience promoting interventions* by individuals with increased vulnerability to stress
- Qualitative and quantitative increase of community studies of novel *glutamate-based behavioral and other lifestyle interventions* aiming to decrease the incidence of stress-related disorders
- Increased number of double-blind placebo-controlled *clinical trials in the field of nutraceuticals, adaptogens, and plant-based foods with glutamatergic properties* for the treatment and prevention of stress-related and neurodegenerative disorders
- More regular use of *stress management and burnout prevention techniques affecting glutamatergic neurotransmission* such as mindfulness meditation and yoga in the occupational settings, including both office and home-based *working environments*

I sincerely hope that this book will inspire you to broaden your personal and professional horizons while helping you to efficiently apply the acquired knowledge in your preclinical and clinical daily practices so you could make a difference in other people's lives.

Enjoy the book and let us meet again in 5 years.

Yours,

Belgrade, Serbia
June 6, 2021

Zoran M. Pavlovic, MD

Acknowledgments

I am very grateful to all those who directly or indirectly supported me in finalizing this part of my 25-year-long "glutamate journey": my **MOTHER NADEZDA** for giving me the idea to start my private psychiatric practice and continuously "indoctrinating" me during my medical studies that psychiatrists are the only "real intellectuals" among medical doctors; my **GRANDFATHER STOJAN**, geologist, member of the Serbian Academy of Sciences and Arts, who obtained his doctorate from the Sorbonne Université in Paris and spent some time during his doctoral studies at the Marie Curie's chemistry lab. He was the first person who showed me certain visually attractive chemistry principles, in his laboratory at the University of Geology in Belgrade, when I was only 4 years old which inspired me to pursue my scientific endeavors later in my life; my **FATHER MILAN** for teaching me how to shave, tie a tie, and about the importance of professional manners and value of friendly relationships with the colleagues along the way; my **DAUGHTER MARIZA** for the initiative to share all her majestic womenswear designs first with me, which consistently boosted my inner creativity beyond medicine and science; my **DOG TOTO** a black French toy poodle for being there for me day and night, always inviting me to play during my daily breaks; my **FRIEND LAMA OLE NYDAHL** for teaching me how to be patient, mindful, and how to share my success and joy with others; **PROFESSOR STUART C YUDOFSKY, MD,** who showed interest to consider my case reports during his tenure as the Chief Editor of *The Journal of Neuropsychiatry and Clinical Neurosciences*, the official journal of the American Neuropsychiatric Association; **PROFESSOR LESLIE CITROME, MD, PROFESSOR SHELDON H PRESKORN, MD, PROFESSOR JUN CHEN, MD, and JULIE ADAMS, MD,** for contributing with their forewords to this edition; **PROFESSOR ERIC HOLLANDER, MD, PROFESSOR PRAKASH MASAND, MD, JOHN DUNLOP, PhD, RAIMUND BULLER, MD**, and **GEORGE GARIBALDI, MD,** reviewers of my first book who motivated me to continue publishing on the topic of glutamate and glutamatergic treatments for psychiatric and neurological disorders;

ALL CHAPTER CONTRIBUTORS of this edition for being diligent, responsive, and always willing to go the extra mile despite the COVID-19 pandemic; and **INA KAREN STOECK, PhD,** Editor at Springer for her insightful suggestions, expert advice, and continuous professional support during the preparation of this manuscript.

Contents

About the Editor

Zoran M. Pavlovic, MD Dr. Pavlovic focuses his work on consulting pharmaceutical and biotech companies on clinical trial design, methodology, biomarkers and surrogate endpoints, patient-reported outcomes, and psychometric assessments related to the development of drug candidates for the treatment of major psychiatric and neurological disorders. His primary interests and expertise have centered on psychopharmacologic approaches to chronic stress, major depressive, anxiety, substance use, neurodegenerative disorders, and chronic pain. Dr. Pavlovic is the Editorial Board Member for *Neuropsychiatric Disease and Treatment* journal and *Innovations in Clinical Neuroscience* and is the author of numerous scientific articles published in most eminent psychiatric, neuropsychiatric, and psychopharmacological journals, such as the official journal of the American Neuropsychiatric Association, official journal of the European Psychiatric Association, and the official journal of the International College of Neuropsychopharmacology (CINP). His first book, *Modulators of Glutamatergic Signaling as Potential Treatments for Neuropsychiatric Disorders,* was published by Nova Science Publishers in 2015.

Chapter 1
Treatments Against Glutamatergic Excitotoxicity in Ischemic Stroke

Andrés Da Silva-Candal, Maria-Perez-Mato, and Jose Castillo

Abstract Brain ischemia is the second cause of death and the first cause of disability in developed countries. Given its high prevalence, there are different therapeutic strategies based on recanalization, neurorepair, and neuroprotection of the tissue. Neuroprotection includes all therapies aimed at reducing cell death after an ischemic process during the acute phase of stroke. The different neuroprotective drugs are classified according to their mechanism of action, being the modulation of the glutamatergic system one of the main targets of neuroprotection. Glutamate is the main neurotransmitter in the central nervous system, where it plays a key role in both development and function. In the brain, glutamate is compartmentalized intracellularly into neurons and astrocytes; and only a small fraction of it extracellularly. During cerebral ischemia, an energy failure occurs that causes an increase in extracellular glutamate, which induces pathogenic mechanisms, leading to neuroexcitotoxicity. In order to mitigate the deleterious effects of excitotoxicity, neuroprotective drugs have been developed to block the postsynaptic and presynaptic receptors of glutamate. These treatments have not shown efficacy in human clinical trials, so the search for new therapeutic strategies is urgent. In line with this, blood/brain glutamate grabbing is well recognized as a novel and protective strategy to reduce the excitotoxic effect of glutamate extracellular excess that accumulates in the brain following an ischemic stroke. Based on this blood/glutamate-grabbing mechanism, the following strategies have been tested: oxaloacetate, glutamate-oxaloacetate transaminase, pyruvate, hemodialysis and peritoneal dialysis, blood glutamate EAAT2-cell grabbing therapy, and riboflavin.

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_1

Keywords Glutamate · Stroke · Excitotoxicity

1.1 Historical Perspective/Introduction

Stroke is a cerebrovascular disease resulting from the disturbance of normal cerebral blood flow, which causes a transient or permanent deficit in the function of one or more parts of the brain. The disturbance of normal cerebral blood flow induces metabolic and cellular changes that can lead to cell death and the disruption of the nervous system. The World Health Organization (WHO) has defined stroke as a rapid clinical development of focal signs of impaired brain function of no apparent origin other than vascular ("The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators," 1988).

Ischemic stroke is the most common type of stroke and represents about the 80% of all stroke cases (Adams Jr. et al. 1993; Arias-Rivas et al. 2012; Del Zoppo et al. 2009; Rodríguez-Yáñez et al. 2008).

In the case of cerebral infarction and attending to the etiology, different ischemic stroke subtypes can be divided (Adams Jr. et al. 1993):

- **Atherothrombotic infarction:** (~20%) usually caused by medium-sized or large infarcts with cortical, subcortical, carotid, or vertebrobasilar topography, in patients with one or more cerebrovascular risk factors. The presence of clinically generalized atherosclerosis, or demonstration of occlusion or stenosis (>50% occlusion or <50% plus two or more vascular risk factors) in cerebral arteries, with an established correlation with the patient's clinic is essential.
- **Lacunar infarction or small vessel disease:** represents ~25% of ischemic strokes, and is characterized by small infarcts (<15 mm in diameter), located in the distribution territory of the penetrating arterioles. Although microatheromatosis and lipohyalinosis of the penetrating arterioles are the most frequent pathological substrate in lacunar infarcts, other less frequent potential causes are cardiac embolism, arterial embolism, infectious arthritis, or prothrombotic state.
- **Cardioembolic infarction:** corresponds to ~20% of ischemic strokes, medium (1.5–3 cm of diameter) or large (>3 cm of diameter) sized infarcts, with symptoms frequently starting during awakening. It is mandatory the presence of a demonstrated embolic origin, and the absence of significant concomitant arterial occlusion or stenosis.
- **Infarction of undetermined etiology**: (~30%) brain infarcts of medium or large size with more than two potential etiologies or unknown origin.
- **Unusual causes** (~5%).

Within focal cerebral ischemia, a distinction can be made between transient ischemic attack (TIA) and cerebral infarction. TIA is defined as focal or monocular cerebral dysfunction with symptoms lasting less than 1 h; the origin is a vascular

insufficiency caused by an arterial thrombus or embolism, associated with arterial, cardiac, or hematologic disease (Sorensen and Ay 2011). Patients with TIA are at increased risk for subsequent major stroke and other vascular events, mainly coronary, and the outcome for each individual is highly variable. Cerebral infarction is defined as injury caused by intense or prolonged ischemia, resulting in irreversible cell loss and neuronal deterioration.

1.2 Biochemistry of Cerebral Ischemia

The acute obstruction of a brain artery induces an instantaneous reduction of blood flow in the corresponding irrigation area (focal ischemia). However, the interruption of the blood supply is not homogeneous and may vary according to the occluded vessel, collaterals, or occlusion type (Castillo 2000).

Within the infarct region two regions can be distinguished: the ischemic core, which is the portion of tissue closest to the affected blood vessel and where ischemia becomes severe, and the so-called penumbra, where the reduction in blood flow is less severe, due to the blood supply from the collateral arteries of the neighboring non-ischemic tissue (Back 1998). Therefore, the severity of stroke will depend on the level of arterial occlusion and the duration of blood flow decrease, making time a very important parameter in this pathology (Fransen et al. 2016). After occlusion and the onset of cerebral ischemia, a series of short- and long-term molecular events will occur. Initially there is an energy failure related to the interruption of oxidative phosphorylation processes and a decrease in adenosine tri-phosphate (ATP) production.

This in turn leads to a failure of the sodium-potassium-ATPase pumps and other ATP-dependent pumps causing the cessation of the transmembrane ionic gradients; this is a key process in the pathophysiological mechanisms of ischemic stroke, with special relevance to cell death in the ischemic core. A few minutes after vascular occlusion (Astrup et al. 1977), neurons and glial cells suffer an extreme depolarization due to the entrance of sodium, chloride, calcium, and water into the cytoplasm (Hansen 1985) and in addition, potassium leaves the cell, inducing a sudden increment of its extracellular levels (Blank Jr. and Kirshner 1977). The energetic failure and the associated ionic changes originate an increment in glutamate, a hyperexcitability of N-methyl-D-aspartate glutamatergic (NMDA) receptors (NMDAr), and of α-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA) receptors (AMPArs), which enhances the initial increase of intracellular calcium (Choi 1987; Choi and Rothman 1990; White et al. 2000) (Fig. 1.1. Self-created image created with Creative commons license).

The intracellular calcium increase does not depend only on the activation of glutamate receptors, but also on the stimulation of voltage-dependent calcium channels. Hyperexcitability causes a depolarization phase at the periphery of the infarct, which raises the energy cost as the membrane attempts to repolarize (Back 1998; Choi 1992; Schiene et al. 1996). Calcium increment, together with acidosis

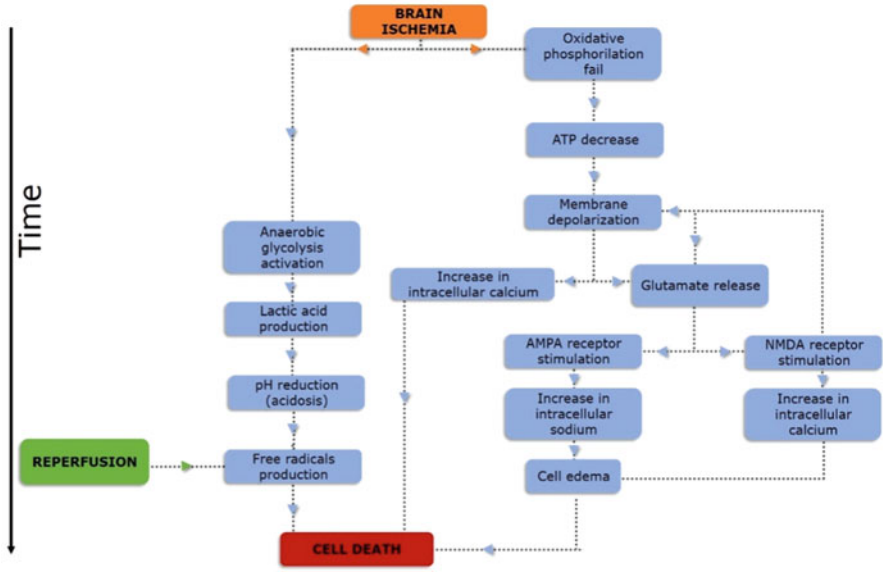

Fig. 1.1 Main molecular events after ischemic stroke that lead to cell death and secondary damage

and peri-infarct depolarization, contributes to initiate the damage. These molecular events are followed by processes of inflammation and apoptotic cell death that contributes to increment the lesion (Banasiak et al. 2000; White et al. 2000). The generation of free radicals occurs during ischemia, and in particular during arterial reperfusion. Free radicals are highly reactive species that are produced in the initial and final phases of cerebral ischemia, following different pathophysiological mechanisms. First, reactive oxygen species are produced by arachidonic acid (AA) metabolism and neuronal nitric oxide (NO) synthase (nNOS) activity. During the intermediate stages, oxygen free radicals are contributed by the infiltration of neutrophils into the ischemic zone. In later stages, they are produced by the synthesis and subsequent activation of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) enzymes (Grandati et al. 1997; Nogawa et al. 1997).

Ischemic cell death, however, can take place in two different ways. The most common is necrosis (McDonald and Windebank 2000), which is the result of the acute energetic failure, mainly located in the core region of the lesion zone, and is characterized by both morphological changes and, ultimately, by cell lysis, which also triggers inflammatory events (Jander et al. 1995). On a different side, apoptotic or programmed cell death, in the region surrounding the core, can be observed when energy-dependent intracellular mechanisms are activated leading to cell damage (Banasiak et al. 2000; Rami et al. 2000).

1.3 Glutamatergic System

Glutamate is the main excitatory amino acid in the mammalian central nervous system. Glutamate plays a key role in cognition, memory, learning, and synapsis. Also, glutamate has a signaling role in endocrine cells as well as in peripheral organs and tissues (Moriyama et al. 2000).

The brain contains a large amount of glutamate, 5–15 mM per kg wet weight, depending on the region (Schousboe 1981), which is mostly distributed in the cellular interior and a tiny portion is in the extracellular space (Boyko et al. 2014). Intracellular glutamate is found in two compartments located in astrocytes and neurons. Astrocytic glutamate is contained in a small pool, which is metabolized to glutamine. In neurons, glutamate is divided in two pools: one in the neuronal soma and dendrites and another in the nerve terminals (vesicles).

During the neurotransmission process, glutamate plays an essential role. The glutamate released by the presynaptic neuron activates the postsynaptic neuron. In addition to neurons, astrocytes are also involved in neurotransmission processes and play a key role in the process of extracellular glutamate uptake. Therefore, it should be noted that glutamate is subject to significant regulation (Cooper et al. 1979; Cooper and Plum 1987; Martinez-Hernandez et al. 1977).

Brain glutamate synthesis occurs mainly through the following pathways:

(a) Glutamate-glutamine cycle
(b) Synthesis in neurons and astrocytes from glucose
(c) Synthesis inside neurons from lactate delivered from astrocytes

Brain glutamate participates in the glutamate-glutamine cycle, but cannot be fully regenerated through this route because glutamate can be oxidized; due to this, glutamate from food does not reach the brain, since it does not cross the blood-brain barrier, making de novo synthesis of glutamate in the brain necessary.

In this regard, it is known that the main substrate for glutamate synthesis is glucose, which is uptaken through GLUT1 and GLUT3 receptors in astrocytes and neurons, respectively (Zou et al. 2010). Through glycolysis, glucose is phosphorylated and pyruvate is generated, which is converted to Acetyl-CoA in the inner mitochondrial membrane. Acetyl-CoA enters the tricarboxylic acid cycle (TCA cycle) and provides α-ketoglutarate (α-KG) as the carbon backbone of glutamate. Neurons can also synthesize glutamate from lactate produced by lactic fermentation in astrocytes. Once synthesized, glutamate is loaded into synaptic vesicles via VGLUTs. In response to neuronal activity, the vesicles, upon interaction with soluble N-ethylmaleimide factor binding protein receptors (SNAREs), fuse with the plasma membrane of presynaptic neurons and release their contents into the extracellular space. Glutamate binds to ionotropic (iGluR) or metabotropic (mGluR) receptors on postsynaptic neurons. In addition to the mentioned receptors, there are specific glutamate transporters called EAATs, which play an essential role in the uptake and maintenance of extracellular glutamate concentrations (Zou et al. 2010; Goncalves et al. 2018).

EAATs are found in astrocytes (EAAT1 and EAAT2) and neurons (EAAT3) (Westbrook 1993). EAATs are antiporters that transport one glutamate molecule along with three Na^+ and one H^+ molecule, while exporting one K^+ molecule (Beretta et al. 2003; Zerangue and Kavanaugh 1996). Glutamate taken up by astrocytes enters the glutamate-glutamine cycle and is converted to glutamine by the action of the enzyme glutamine synthetase (a specific enzyme of astrocytes and oligodendrocytes). This glutamine is released from astrocytes via the N-system glutamine transporter (SN1) and reaches neurons via system A transporter (SAT1). Here, glutamine is converted to glutamate via phosphate-activated glutaminase (Fremeau et al. 2004). Astrocytic glutamate that is not converted to glutamine is degraded to α-KG and enters the TCA cycle.

In addition to the involvement of astrocytes and neurons in extracellular glutamate uptake, many studies indicate that endothelial cells (ECs) play a key role in the maintenance of extracellular glutamate concentration. The presence of EAATs in the abluminal membrane of brain ECs and their ability to uptake and accumulate glutamate have been described. Glutamate accumulated in the ECs can undergo different processes, from its transport into the bloodstream to its metabolization (Zerangue and Kavanaugh 1996). In vivo studies show that glutamate can be transported into the bloodstream via a facilitatory glutamate transporter XG- present in the luminal membrane (Oldendorf and Szabo 1976). In addition, glutamate can be metabolized in EC mitochondria, where it is converted to α-KG. From there, α-KG is converted to pyruvate, which can be converted to lactate in the cytosol and transported via MCT-1 on the luminal membrane into the blood (Cederberg et al. 2014). Endothelial cells, via transporters located in the abluminal membrane, are able to uptake glutamine from the extracellular space. Glutamine is hydrolyzed to glutamate and NH4+ by glutaminase present in the cytoplasm of ECs (Hawkins 2009).

The main pathways of glutamate synthesis and metabolization are represented in Fig. 1.2. Image adapted from Castillo et al. and created with Servier Medical Art.

1.4 Molecular Components of the Glutamate Neurotransmitter System as Potential Drug Targets: Receptors and Transporters

Glutamatergic neurotransmission serves as excitatory relay stations between presynaptic nerve terminals and postsynaptic dendritic. The glutamatergic synapse plays a crucial role in a wide range of normal physiological functions and involves a series of transporters and receptors.

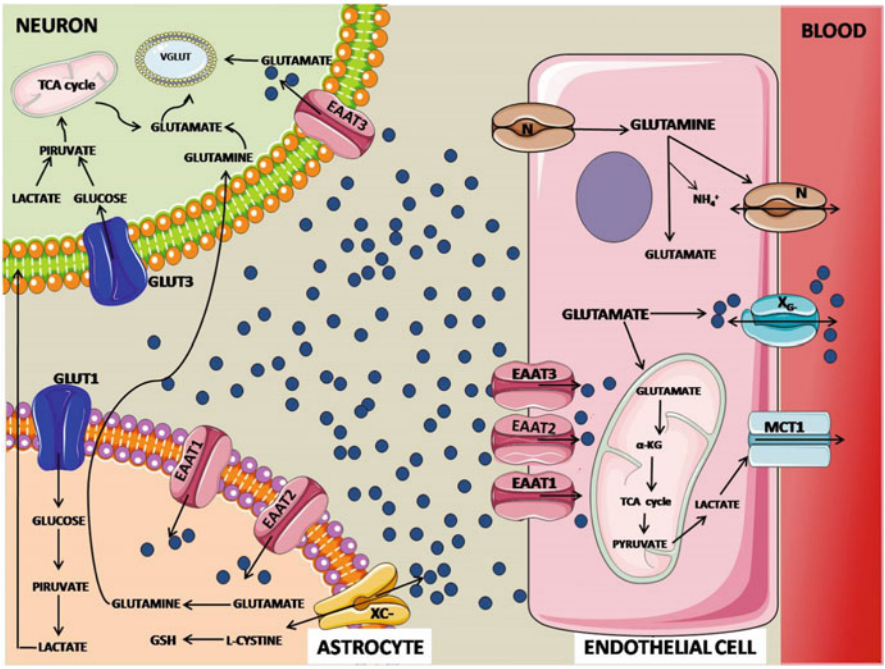

Fig. 1.2 Main routes of glutamate synthesis and metabolization

1.4.1 Glutamate Transporters

1.4.1.1 Excitatory Amino Acid Transporters (EAATs)

EAATs are polypeptides of 500–600 amino acid residues and transporters consisting of six to eight putative transmembrane domains, one or two re-entrant loops and cytoplasmic N- and C-termini (Shigeri et al. 2004). To date, five subtypes (EAAT1-5) have been described, with amino acid homology of 50–60%. The main function of EAATs is the transport of glutamate from the extracellular space across the plasmatic membrane. This involves the co-transport of glutamate and three Na^+ and one H^+ ion, which causes a conformational change of the transporter and the subsequent release of the charge into the cell cytoplasm. Subsequently, a K^+ ion binds to the EAAT transporter, and returns to its original conformation with the subsequent release of K^+ ion (Krzyzanowska et al. 2014).

EAATs (1–3) are mainly expressed in the central nervous system (CNS), while $EAAT_4$ and $EAAT_5$ are in the cerebellum and retina, respectively (Krzyzanowska et al. 2014). $EAAT_1$ and $EAAT_2$ are found in astrocyte membranes, with the highest densities of both in the membrane of astrocytes overlooking the neuropil. $EAAT_1$ is predominantly expressed in astrocytes, while $EAAT_2$ is mostly expressed in astrocytes, but has also been identified (about 10%) in hippocampal nerve terminals. $EAAT_3$ is selective for neurons, mainly in dendrites and cell bodies, and its

expression levels are lower than the detected levels of $EAAT_2$ (Zhou and Danbolt 2000). In addition to the localization described above, different studies indicate that EAATs are present in the endothelial cells of the blood-brain barrier (Cederberg et al. 2014), where they participate in the cellular mechanism for brain glutamate efflux (Castillo et al. 2016).

The main function of EAATs is to regulate the extracellular concentration of glutamate and to maintain it at low physiological levels to avoid its deleterious effects. After releasing into the synaptic cleft, glutamate is rapidly uptaken via EAATs (1–3) into glial cells and neurons. $EAAT_2$ is primarily responsible for more than 90% of the total glutamate uptake (Maragakis et al. 2004). The function of $EAAT_4$ is to regulate neuronal excitability through regulation of neuronal depolarization (Krzyzanowska et al. 2014). $EAAT_5$ mediates the light response of depolarizing bipolar cells (DBC) in dark-adapted mouse retina (Tse et al. 2014).

The regulation of extracellular glutamate levels is essential for the maintenance of normal brain function. Deficiencies in this process are linked to several neurological diseases, such as cerebral ischemia, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. Given that the $EAAT_2$ transporter is the main regulator of the homeostasis of glutamate levels in the brain, proper expression and regulation of $EAAT_2$ is critical for maintaining extracellular glutamate levels and the survival of neurons. The critical role of $EAAT_2$ in the regulation of glutamate in different diseases suggests that this transporter can be used as a target for the development of new molecules capable of regulating its function, both in vitro and in vivo, with ultimate applications in humans. In this regard, β-lactam antibiotics have been identified as transcriptional activators of $EAAT_2$. Studies with β-lactams showed that they are able to facilitate glutamate uptake by astrocytes providing neuronal protection. This finding suggests that these drugs have potential applications as therapeutic agents to limit and prevent glutamate excitotoxicity. For this reason, further study of the mechanism (s) underlying EAAT2 transcriptional activation may help in the discovery of new drugs to improve the prognosis of diseases with a glutamate excitotoxic component (Kim et al. 2011).

1.4.1.2 Vesicular Glutamate Transporters (VGLUSTs)

VGLUTs are responsible for transport of glutamate into the synaptic vesicles. The vesicular uptake is dependent on a proton gradient that they create by hydrolyzing ATP with H^+-ATPase. This enables the flow of H^+ into the interior of the synaptic vesicle making it more acidic and generating a pH gradient across the vesicle membrane (Krzyzanowska et al. 2014).

The vesicular glutamate transporters are polypeptides consisting of about 600 amino acid residues. Three subtypes of VGLUTs (1, 2, and 3) have been identified and appear to share more than 70% homology. The transmembrane topology of VGLUTs is thought to consist of 8–10 putative transmembrane domains. A highly conserved glycosylation site between transmembrane domains

1 and 2, and numerous consensus sequences for phosphorylation by various protein kinases are also predicted (Shigeri et al. 2004).

The isoforms VGLUT1 and VGLUT2 are expressed mainly in glutamatergic neurons and their expression in CNS seems to be largely complementary with only a limited overlap. VGLUT1 is localized in the neocortex, hippocampus, and amygdale. VGLUT2 can be observed in olfactory bulb, cerebral cortex, dentate gyrus, thalamus, and hypothalamus. VGLUT3 is localized in a limited number of glutamatergic neurons in multiple brain regions: neocortex, hippocampus, olfactory bulb, hypothalamus, substantia nigra. Additionally, VGLUT3 has been found in hippocampal and cortical GABAergic neurons (Krzyzanowska et al. 2014).

1.4.1.3 The Glutamate-Cystine Exchanger (xCT)

Glutamine-cystine exchangers (xCT) act as a cystine transporter that uses the transmembrane gradient of glutamate as driving force. It is a heterooligomer consisting of two different subunits: the 4F2hc surface antigen (slc3a2), the xCT protein (slc7a11). xCT does play a role in glutathione production and has been suggested to be a major source of extracellular glutamate. Although the distribution of xCT in the brain has not yet been definitively determined, the available data suggest its levels are low (Zhou and Danbolt 2000).

1.4.1.4 Intracellular Glutamate Carriers

When glutamate enters the cytoplasm, it may undergo further redistribution to mitochondria through mitochondrial glutamate transport. There are in fact in the mitochondria four different carriers, AGC1, AGC2, GC1, and GC2 (Zhou and Danbolt 2000) that serve for glutamate translocation.

1.4.2 Glutamate Receptors

Receptors can be divided into two broad categorizations, ionotropic and metabotropic receptors; Ionotropic glutamate receptors are ion channels that flux cations (Ca^{2+}, Na^{+}) while metabotropic receptors, on the other hand, activate or inhibit second messenger systems via interactions with cognate G-proteins.

1.4.2.1 Ionotropic Glutamate Receptors

Three classes of ionotropic glutamate receptors have been identified: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid

(AMPA), and kainate (KA). Ionotropic glutamate receptors form tetrameric complexes of individual/heteromeric subunits.

- **NMDA Receptors**: NMDA receptors have been reported to have a high affinity for glutamate. Three families of NMDA receptor subunits have been identified: NR1, NR2AD, and NR3A-B, which are expressed in different brain areas. In this regard, NR1 expression is known to be of vital importance in the brain, as it is involved in neurodevelopmental pathways. Previous studies have shown that NR1 knockout mice die shortly after birth due to respiratory failure. NR2A expression is localized in the neocortex and hippocampus, while NR2B is localized in the forebrain. However, NR2C and NR2D are mainly expressed in the cerebellum and diencephalon. NR3A is predominantly located in the neocortex and its function is linked to the regulation of neurodevelopment. In contrast, NR3B is expressed in the brainstem and alpha motor neurons of the spinal cord. Recent research places NR3B in the cerebellum and hippocampus.

  NMDA receptors are tightly regulated in the mammalian brain and are the only receptors that require co-agonists for activation. At least six sites have been described as binding sites for the following NMDA receptor ligands: short-chain dicarboxylic amino acids (glutamate, aspartate), polyamines, and cations (Mg^{2+}, Zn^{2+}, and H^{+}) that regulate ion channel opening. Glutamate and several competitive NMDA receptor antagonists such as D-2-amino-5-phosphonopentanoic acid (D-AP5) and 3-(2-carboxypiperazin-4-yl)1-propeni-1-phosphonic acid (2R-CPPene) bind to the NR2 subunit of the tetrameric receptor complex. Extracellular Mg^{2+} acts as a voltage-dependent "pore blocker" and prevents cation efflux. Zn^{2+}, a divalent cation, acts as an allosteric modulator of glutamate receptors across synaptic vesicles. Hydrogen ions (H^{+}) also act as allosteric modulators of glutamate receptors. H^{+}, at physiological pH, reduces the frequency of channel opening due to H^{+} binding to NR2B. Polyamines have a pH-dependent receptor modulatory role. The function of polyamines (spermine, spermidine) in binding to the channel is to unblock it and thus increase cation flux (Niciu et al. 2012).
- **AMPA/Kainate Receptors**: AMPA/Kainate receptors are widely distributed in the mammalian CNS and are responsible for mediating the glutamate binding-dependent excitatory response. AMPA receptors are composed of GluR1-4 subunits and kainate receptors of GluR5-7 and KA1-2 subunits. GluRs differ from other amino acid and monoaminergic neurotransmitter receptors in that they contain a longer extracellular N-terminus than usual. Glutamate release at the synaptic cleft elicits excitatory postsynaptic potentials (EPSPs). AMPA receptors mediate a rapid rise and decay of the current, while NMDA receptor activation provides a more sustained phase of depolarization that can last for several hundred milliseconds. The binding of glutamate to NMDA/AMPA receptors explains the pharmacokinetic differences: prolonged receptor activation results in slower dissociation of agonist and receptor.

  The synthesis of AMPA receptors occurs in the soma and their translocation to the cell membrane occurs via the secretory pathway, which involves multiple

steps of membrane sorting and cytoskeletal transport proteins. The transport of AMPA receptors to the cell surface and their involvement in the synapse is regulated by two mechanisms: exocytic and endocytic trafficking and recycling, respectively, in the secretory pathway and membrane diffusion from extrasynaptic to postsynaptic locations. Several studies have proposed that trafficking and surface diffusion of AMPA receptors plays an essential role in learning and memory. Other regulators of AMPA receptor expression are stress hormones, which promote inducing and suppressing mechanisms of synaptic plasticity and cognition. The regulation of ionotropic glutamatergic neurotransmission is very complex, due to the wide molecular variability that regulates this process at the transcriptional and post-transcriptional level (Niciu et al. 2012).

1.4.2.2 Metabotropic Glutamate Receptors

Metabotropic receptors have a very different mechanism of action with respect to ionotropic glutamate receptors, which are dependent on cation efflux metabotropic glutamate receptors and exert their function through the recruitment and activation of intracellular trimeric G-proteins and downstream signal transduction pathways. Metabotropic glutamate receptors, like all G protein-coupled receptors, are seven transmembrane domain receptors with a particularly long intracellular C-terminal and extracellular N-terminal, a feature similar to AMPA receptors. Metabotropic receptors are primarily expressed in perisynaptic and extrasynaptic areas of neurons and glial cells. The function of these receptors is related to the modulation of synaptic activity and plasticity. Eight types of metabotropic glutamate receptors (mGLuR1-8) have been described: group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3), and group III (mGluR4-8). This division is made on the basis of amino acid homology, agonist binding, and activated signal transduction cascades. Group I receptors exert their action through two pathways: phospholipase C via inositol-1,4,5-trisphosphate (IP3) to release Ca2+ from intracellular stores and diacylglycerol (DAG) to stimulate protein kinase C. Group II and group III metabotropic receptors are coupled to inhibitory G-proteins (Gi) that decrease intracellular cyclic adenosine monophosphate (cAMP) through inhibition of the adenylyl cyclase/protein kinase A pathway (Niciu et al. 2012).

Glutamate, with different degrees of affinity, binds to metabotropic receptors and triggers their activation. Studies have shown that postsynaptic activation of metabotropic receptors modulates ion channel activity, which is dependent on the signal transduction cascade (Niciu et al. 2012). Metabotropic receptors located on presynaptic membranes decrease both excitatory glutamatergic and inhibitory GABAergic neurotransmission. Although the specific mechanism mediating presynaptic modulation is unclear, most studies suggest that metabotropic glutamate receptors elicit their effects through modulation of presynaptic voltage-dependent Ca^{2+} channels. Currently, many research efforts are focusing on the development of positive and negative modulators of presynaptic group II and III metabotropic receptors, with the aim of using them in neuropsychiatric diseases. There are also

other strategies focused on modulating the activity of group I metabotropic receptors (Niciu et al. 2012).

1.5 Current Clinical Treatments

Nowadays, in clinical practice there are two major approaches in the acute treatment of ischemic stroke, both based on the recanalization of the occluded vessel in order to reperfuse the tissue area affected, pharmacological or mechanical (thrombectomy) thrombolysis, which are the strategies that report higher benefits for the patient, in terms of neurological outcome. The only authorized thrombolytic treatment for the use in the brain is the recombinant tissue plasminogen activator or rt-PA, and enzyme involve in the clot degradation of the occluded vessel. The thrombectomy is a technique which allows the extraction of the thrombus by a mechanical device. Both therapies have pushed for the creation of stroke units inside hospitals, which have improved the management of stroke patients together with a better control of the prognostic factors. Nevertheless, a low number of stroke patients are currently treated by these procedures in most developed countries. Such reduced numbers may be due to different factors, including the narrow therapeutic window and the high risks of hemorrhage transformation. Although the main objective after recanalization is the regulation of prognostic factors as: oxygen, arterial tension, temperature, or glycemic levels, current neuroprotective strategies are required to work at both stages, by widening the therapeutic window and by reducing the associated risk factors (Adams et al. 2007; Dirks et al. 2011; Donnan et al. 2011; Lees et al. 2010; Wahlgren et al. 2007). The therapeutic window associated with intravenous thrombolytic treatment is 4.5 h. The extension of this window would be possible by selecting candidate patients with a large penumbral area (area of the brain susceptible of damage if it is not resolved within the first 12–24 h (Rodriguez-Yáñez et al. 2011).

Neuroprotection is a term that conglomerates a variety of strategies focused on reducing cell death after an ischemic event, without affecting tissue reperfusion. So far, several compounds have been proposed to block the pathway leading to ischemia-induced cell death at different steps of the ischemic cascade. Most of these compounds have shown positive effects in experimental studies, although unfortunately none of them have shown beneficial effect in clinical trials (Gladstone et al. 2002; Kaur et al. 2013).

1.5.1 Glutamate Receptors Modulation

As previously explained, glutamate is the major excitatory CNS neurotransmitter, also capable of inducing excitotoxic neural injury in the setting of cerebral ischemia and other disorders. Glutamate and related excitatory amino acids interact with

several receptor-classes, which are relevant in neuroprotection. These include the NMDA and AMPA receptors (Ginsberg 2009).

It has been demonstrated that the modulation of NMDA receptors as well as their blockade by means of antagonists reduces infarct size and neurological deficit in animal models of focal cerebral ischemia. However, its clinical use has presented several side effects, especially cardiovascular and psychiatric effects. The NMDA receptors are heterotetramers composed by two GluN1 subunits and two GluN2 subunits. This GluN2 can be divided into GluN2 A, B C, or D, as well as GluN3A and GluN3B. The anatomical distribution of these receptors is heterogeneous and entails some variability in the treatments targeting glutamate receptors. Due to the importance of the excitotoxicity mediated by these receptors, the first treatments developed were focused on the blockade by means of different mechanisms. One of the first approaches for NMDA blockade was the use of Dizocilpine; this treatment showed promising results in preclinical stages, however histological alterations observed in rats prevented its passage to clinical trials (Olney et al. 1989). *Selfotel*, a competitive antagonist of NMDA receptors showed improvement of outcome and no significant increase of mortality in a phase III study, but a high incidence of psychiatric adverse effects conditioned its withdrawal from clinical phases. Likewise, *Dextromethorphan* and its metabolites *Dextrorphan* and *Aptiganel* were discontinued by an unfavorable risk-benefit balance and numerous side effects. *Eliprodil* reduces the action of glutamate by interfering with the sensor polyamine site on the NMDA receptor but showed no difference with placebo. With *Gavestinel*, antagonist of the NMDA glycine receptor, two large phase III trials were planned and completed and both were neutral: showed excellent tolerance but no efficacy (Jia et al. 2015). Magnesium sulfate has been also tested as a non-competitive inhibitor on glutamate receptors, but its clinical efficacy has not been demonstrated so far (Muir et al. 2004; Singh et al. 2012).

On the other hand, several AMPA antagonists showed neuroprotective efficacy in preclinical studies of both focal and global cerebral ischemia (Ginsberg 2009). In patients, YM872, an AMPA antagonist, was subjected to a dose-escalation study showing good tolerance in young and elder patients (Akins and Atkinson 2002). Other antagonists like ZK200775 worsened the neurological condition in patients with acute ischemic stroke.

Continuing with this line, a secondary approximation has been the blockage of the calcium channels involved in the excitotoxic cascade initiated with glutamate.

1.5.2 Calcium Blockers

Calcium plays an important role in stroke pathophysiology. The blockage of calcium channels stops neuronal calcium intake, hence reducing cell death. An example of this family of compounds is *Nimodipine, a* is highly lipophilic molecule able to cross the blood-brain barrier, and reach brain and cerebrospinal fluid. Over 250 animal studies of *nimodipine* in cerebral ischemia have been published, but only 10 of these

studies reported a positive outcome (Kaur et al. 2013). However, none of the members of this family of compounds have demonstrated a clear neuroprotective activity on clinical trials; furthermore, highest doses of these treatments are related with a bad outcome (Zhang et al. 2012).

1.5.3 Other Downstream Modulations of Glutamate Receptors

The activation of NMDA and AMPA receptors after glutamatergic overstimulation is accompanied by heterogeneous signals that lead to the generation of secondary damage and promote cell death. Different points of these signaling pathways have been chosen as the main therapeutic targets in ischemic stroke. The overactivation of the NMDA receptors leads to an increase in the expression of the nNOS enzyme that generates an increase in NO. NO reacts with superoxide free radicals to form the highly reactive oxidant peroxynitrite (Radi et al. 1991). This may lead to protein oxidation, lipid peroxidation or DNA damage that increases cell death (Lipton et al. 1993). One of the protagonists in the increase of NO is the postsynaptic density-95 protein (PSD95) linked to NMDA receptors GluN2B and to the enzyme nNOS, respectively (Kornau et al. 1995). The existence of this union between receptor and NO allows the development of treatments that block the intermediate signaling by slowing down the associated oxidative stress. The NA-1 peptide stands out, which decouples the union between the receiver and PSD95 preventing the signalization process from continuing. Favorable results in preclinical studies with non-human primates promoted the development of a proof of concept clinical trial in humans (Cook et al. 2012). This study enrolled one hundred and eighty-five patients who randomly received either NA-1 or saline control. NA-1 treatment reduced the number and volume of strokes by all MRI criteria and improved neurological outcome. A very similar approach is the one developed by the peptides ZL006 and IC87201 which, in this case, interrupt the signaling between PSD95 and nNOS. The use of this molecules have only been validated in preclinical studies, showing a diminution of the ischemic injury and granting a better specify with no affection of the normal nNOS activity in neurons.

Apoptosis is another of the pathways studied in brain neuroprotection. Damaged cells initiate a signaling process that ends with the programmed death of neurons increasing secondary damage. Many of these processes are dependent on intracellular Ca^2, one of the consequences of overstimulation of NMDA receptors. One of these apoptotic effectors is death-associated protein kinase 1 (DAPK1) (Bialik and Kimchi 2006; Wang et al. 2017). The cell death-inducing activation of DAPK1 largely depends on its intrinsic kinase activity (Cohen et al. 1997). Under ischemic conditions the overactivation of GluN2B NMDAR leads to excessive Ca2+ influx into the cell and activates the calmodulin and the calcineurin phosphatase (CaN), which in turn dephosphorylate and activate DAPK1 promoting neuronal loss.

Various treatments have been developed to interrupt this signaling pathway, such as the peptide Tat-GluN2B$^{CT1292–1304}$ in order to uncouple the DAPK1 from the glutamate receptor, decreasing the Ca + 2 influx and increasing cell survival (McQueen et al. 2017; Tu et al. 2010). On the other side, DAPK1 has been told to modulate p53 activation leading to a pro-apoptotic signaling cascade. In this regard, an interfering peptide Tat-p53$^{DM241–281}$ was constructed to disrupt the interaction between DAPK1 and p53, showing reduction of infarct volume with a long therapeutic window in preclinical models of ischemic stroke (Wang et al. 2014).

1.6 Future Developments and Perspectives Related to Glutamate Modulating Drugs

High glutamate concentration at the synaptic cleft is rapidly (up to 1000 fold) reduced by the action of glutamate transporters present on both nerve terminal and surrounding astrocytes to prevent glutamate excitotoxicity (Danbolt 2001). There is an unfavorable gradient between brain (1–10μM) and blood (40–60μM) glutamate concentration into the EC (O'Kane et al. 1999); when endothelial glutamate concentration becomes higher than the blood glutamate concentration, glutamate is transported into the blood by a mechanism that facilitates blood excretion of glutamate from the brain. The presence of EAATs in the blood-brain barrier and their ability to accumulate large intracellular glutamate concentrations started the hypothesis that lowering blood glutamate levels could increase the concentration gradient from endothelium to blood and thereby increase the elimination of glutamate from the brain; this is known as the blood glutamate-grabbing hypothesis (Fig. 1.3). To demonstrate this glutamate-grabbing hypothesis, the blood resident enzyme glutamate-oxaloacetate transaminase (GOT), which transforms glutamate into α-ketoglutarate and aspartate in the presence of oxaloacetate, was used. This enzyme when oxaloacetate is artificially increased shifts the equilibrium of the reaction to the right side, thereby decreasing glutamate levels in blood. In this sense, Gottlied et al. (2003) demonstrated that when radioactive glutamate was

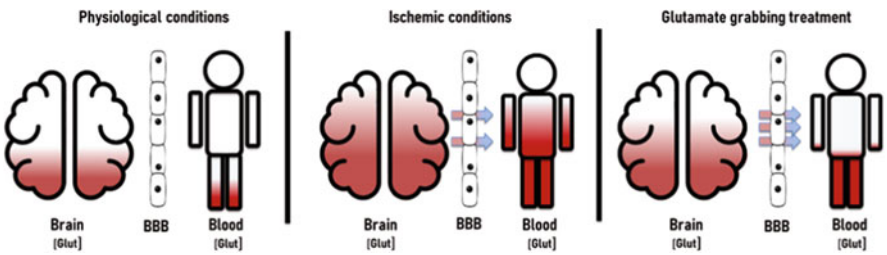

Fig. 1.3 Basis of treatment with glutamate grabbers. The treatment decreases the systemic levels of glutamate increasing the washing of glutamate excess generated after cerebral ischemia decreasing the associated damage

injected into the lateral ventricles in experimental animals the oxaloacetate induced a decrease in blood glutamate levels followed by an increase of the diffusion of radioactive brain glutamate into the blood. Similar effects were observed in other studies using two microdialysis probes, one infusing and the other collecting glutamate; oxaloacetate treatment reduced the rate of radioactive glutamate collection by the second probe.

Alternatively, malate pretreatment, a GOT blocker, has also demonstrated inhibition of the oxaloacetate-dependent lowering of blood glutamate, confirming that the effect of oxaloacetate on blood glutamate levels was mediated by blood glutamate lowering.

These studies inspired several investigations of the effects of blood glutamate grabbing during pathological conditions such as ischemia, subarachnoid hemorrhage, closed head injury, traumatic brain injury, and paraoxon intoxication. The studies utilized different approaches to reduce blood glutamate levels. All these studies came to the same general conclusion: lowering blood glutamate levels decreases the morbidity of the disease states, for instance, through better recovery, better neuron survival, or smaller stroke volumes (Cederberg et al. 2014). The blood glutamate grabbers show potential for the development of novel, effective, and safe therapeutic agents. Whereas NMDAr antagonists were ineffective or potentially harmful, blood glutamate grabbers do not act on glutamate receptors nor do they interfere with normal cellular signaling processes. Their action is only in the blood, and they accelerate a physiological mechanism of removing glutamate only from areas in which glutamate is pathologically elevated (Boyko et al. 2014). Based on mechanism of blood glutamate grabbing, the following strategies have been tested:

1.6.1 Oxaloacetate (OxAc)

The first studies showing the neuroprotective effect of OxAc on cerebral ischemia were observed in rats subjected to photothrombotic lesions. This effect was later corroborated in a model of ischemia induced by transient middle cerebral artery occlusion (MCAO). In this study, different doses of OxAc were used to evaluate the effect of OxAc on blood glutamate depletion. Following STAIR criteria, an intravenous bolus injection of OxAc (effective dose used of 3.5 mg/100 g, animal weight) was administered 90 min after occlusion, which resulted in a decrease in blood glutamate levels, followed by a reduction in infarct volume and edema after ischemia. These effects were correlated with reduced motor deficits. Spectroscopic analysis determined that the increase in brain glutamate observed in control animals after MCAO was reduced in those animals treated with OxAc, confirming that the neuroprotective effect was associated with decreased brain glutamate levels (Campos et al. 2011a, b). Other studies on models of cerebral ischemia showed that OxAc-induced reduction in blood glutamate levels was inhibited in the presence of excess serum glutamate or when administered in combination with malate (a GOT

inhibitor), effectively demonstrating its serum glutamate scavenger action (Castillo et al. 2016; Teichberg et al. 2009).

In addition to the protective effects of OxAc related to glutamate reduction, other protective mechanisms have been proposed. In this regard, it is known that this molecule is involved in energy metabolism and provides antioxidant protection to cells under stress, such as hydrogen peroxide, so it has been suggested that the protective role of OxAc is also associated with mechanisms other than serum glutamate reduction.

Regarding the use of OxAc as a treatment for serum glutamate uptake in cerebral ischemia, the main limitation is associated with the high doses that are likely to be necessary in patients to obtain the same effects as those observed in experimental animals. In this regard, OxAc has already been used in humans and no toxicity has been reported. In particular, a study published in the 1960s used OxAc (200–1000 mg a day in three divided doses administered orally) to treat diabetic patients, and reported no effect on liver function or blood levels of acetone and cholesterol at the doses administered. Therefore, we can conclude that, to date, there are no studies describing evidence of toxicity from the use of OxAc (Yoshikawa 1968).

1.6.2 Glutamate-Oxaloacetate Transaminase (GOT)

Clinical studies have shown that patients with a good prognosis have lower glutamate levels and higher GOT levels in blood samples collected on admission. Therefore, a significant inverse correlation between GOT and glutamate levels was described. Increased blood GOT levels are related to a good functional prognosis and reduced infarct volume. These results support the idea that GOT could metabolize blood glutamate and increase the glutamate concentration gradient from the brain parenchyma to the circulation (Campos et al. 2011a, b).

In view of the above, GOT is postulated as a potential blood glutamate scavenger agent. In this regard, a study was carried out using recombinant human GOT1 (rGOT1) in the rat model of transient MCAO. The results showed that administration of GOT induced a reduction in serum and brain glutamate levels, leading to a reduction in infarct volume and an improvement in sensorimotor deficits. In this study, the optimal dose of rGOT required for maximal blood glutamate reduction was tested in a dose-response assay. These experiments showed that a dose of 12.88 mg per 100 g (animal weight) was the most appropriate. To determine whether the effect of the enzyme could be potentiated by OxAc (co-substrate of the enzyme reaction), the rGOT1 dose (12.88 mg/100 g) was administered with a non-effective dose of OxAc (1.5 mg/100 g); it was observed that the protective effect of rGOT1 was increased with this new strategy, indicating that co-administration of rGOT1 and OxAc may be the most effective therapy for serum glutamate uptake. The administration of an endogenous serum enzyme such as GOT1 as a new protective treatment in cerebral ischemia is a promising strategy, since there will be no toxic effects from

rGOT1 administration in humans, as levels of this enzyme vary among healthy human subjects (7–45 U/l), and it has been shown to increase >10-fold in patients with liver damage (Perez-Mato et al. 2019).

The main hypothesis to explain the observed therapeutic effect of the OxAc and GOT combination may be due to the fact that the endogenous OxAc concentration may become a limiting factor of the enzymatic reaction when GOT activity increases after treatment. Therefore, treatments based on the combined administration of rGOT1 supplemented with a low concentration of OxAc lead to a rapid and sustained reduction in serum glutamate concentration. This makes them the optimal combination to achieve the maximum protective effect in ischemia, as the low dose of OxAc is sufficient to increase the glutamate binding activity of rGOT1, which reduces the potential complications associated with a high dose of this molecule, as previously described (Castillo et al. 2016).

1.6.3 Pyruvate

The use of an enzymatic approach involved in glutamatergic metabolism, as previously described, opens a new range of possibilities for the glutamate grabbing treatment of ischemic stroke. Similar to the mechanism of action of oxaloacetate, pyruvate has also been described as an effective drug capable of reducing blood glutamate levels. Pyruvate induces an activation of the blood resident enzyme glutamate-pyruvate transaminase (GPT); this enzyme catalyzes the reversible reaction of pyruvate and glutamate to alanine and a-ketoglutarate. Thus, when an ischemic event occurs with the consequent increase in serum glutamatergic levels, the administration of pyruvate as a treatment shifts the equilibrium of the reaction to the opposite side, transforming the excess glutamate and pyruvate administered into a decrease in serum levels. Similar to previous studies with GOT, GPT levels were associated with lower levels of blood glutamate, better functional outcome, reduced infarct volume, and lower percentages of early neurological deterioration, although this association was stronger for GOT than GPT levels (Boyko et al. 2011; Castillo et al. 2016; Zlotnik et al. 2007).

1.6.4 Hemodialysis and Peritoneal Dialysis

Another potential beneficial use of serum glutamate depletion is based on a non-pharmacological approach, blood and peritoneal dialysis. This method applies the principles of diffusion and osmosis across the membrane to establish fluid and electrolyte balance in patients with pathologies as acute renal failure and end-stage renal disease. In this regard it has been demonstrated patients with end-stage renal failure undergoing hemodialysis showed higher blood glutamate concentrations compared to healthy controls. During hemodialysis (especially in the first hour),

glutamate concentrations decreased regardless of filter pore size, blood flow rate, or sex (Brotfain et al. 2018). Similarly, peritoneal dialysis caused a decrease in blood glutamate with a corresponding increase in glutamate in the dialysis solution (Rogachev et al. 2012). In preclinical research in a rat model of stroke, the reduction in blood glutamate levels observed with peritoneal dialysis was associated with a decrease in infarct area.

The development of an extracorporeal technique opens new ways in stroke treatment. Likewise, a non-pharmacological therapeutic approach suggests an advantage due to the lack of important side effects, as may be the case with other drugs, and which in many cases would limit translationality from basic to clinical research. Through an alternative pathway, these studies provided that blood glutamate reduction is an effective protective therapy strategy and can also be used as supporting evidence that hemodialysis and peritoneal dialysis can be an additional efficacious modality to reduce blood glutamate concentration, as demonstrated in preclinical studies (Godino Mdel et al. 2013). This hypothesis is subjecting to phase IIa clinical trial (EudraCT Number: 2012-000791-42). (EudraCT Number: 2012-000791-42) (Castillo et al. 2016; Rogachev et al. 2012).

1.6.5 Blood Glutamate EAAT2-Cell Grabbing Therapy

In addition to the therapies previously described, a study that has recently been published proposed a new strategy based on the induction of the expression of the $EAAT_2$ transporter in mesenchymal stem cells (MSCs). Both the EAAT2 transporter and the MSCs have key roles in cerebral ischemia: the $EAAT_2$ transporter plays a fundamental role in glutamate reuptake and homeostasis of the same, and MSCs are considered the most promising candidates for stem cell therapy against ischemic stroke owing to their intrinsic capability to secrete growth factors and immunomodulatory cytokines. With this in mind, the combination of the $EAAT_2$ transporter and MSCs allowed the development of a cell-based glutamate-grabbing therapy for systemic administration, combining the intrinsic properties of these cells with the excitotoxic protection (Perez-Mato et al. 2019).

In order to carry out this study, $EAAT_2$-encoding cDNA was expressed into MSCs by electroporation. $EAAT_2$ expression and functionality were evaluated by *in vitro* assays. Blood glutamate-grabbing activity was tested in healthy and ischemic rat models treated with 3×10^6 and 9×10^6 cells/animal. In addition, reduction of infarction volume and functional recovery were evaluated in ischemic animals. The results showed that the expression of $EAAT_2$ in MSCs conferred the expected glutamate-grabbing activity *in vitro* and *in vivo* studies. Unexpectedly, both cell doses of non-transfected MSCs induced higher protection than transfected $EAAT_2$–MSCs by another mechanism independent of the glutamate-grabbing capacity (Perez-Mato et al. 2019).

The interpretation of these results led them to think that the transfection technique used could be altering some of the anti-inflammatory capacities, which had not been

previously studied, associated with MSCs. In order to study this, the authors evaluated the secretion of IL-6, the main pro-inflammatory molecule, in electroporated MSCs. The results showed that the transfection process triggered the release of IL-6 in MSCs and that animals treated with transfected EAAT2-MSCs had higher IL-6 levels than animals treated with non-transfected MSCs. Although the transfection procedure most likely interferes with some of the intrinsic protective mechanisms of mesenchymal cells, the results showed that the induced expression of $EAAT_2$ in cells represents a novel alternative to mitigate the excitotoxic effects of glutamate and paves the way to combine this strategy with current cell therapies for cerebral ischemia.

1.6.6 Riboflavin as a New Glutamate Grabber: Preclinical and Clinical Validation

As previously described, the efficacy of glutamate grabbers has been widely demonstrated through various strategies and approaches. However, the nature of some of these approaches limits their clinical translationality, and it is imperative to demonstrate the efficacy of this strategy in humans. For this purpose, a recent work analyzed, chemically and pharmacologically, 1120 diverse compounds (ca. 90% being FDA approved drugs) from the Prestwick Chemical Library (PCL) (http://prestwickchemical.com/prestwick-chemical-library.&html) regarding their capacity as glutamate grabbers in a high throughput screening (HTS). The hit with the best results was riboflavin. Riboflavin or vitamin B_2 is a component of water-soluble vitamins; its supplement is authorized for use in patients and secondary effects have not been reported. This compound was subjected to a dose-response study in healthy animals. An effective dose of 1 mg/kg was established, significantly reducing plasma levels of glutamate at 30 min after intravenous administration. This compound demonstrated a reduction of infarct volumes in animal model MCAO accompanied by a systemic reduction of glutamate levels. As possible treatment in hyperacute stages prior to identification by image of the hemorrhagic or ischemic nature of the stroke, the drug was administered to rats subjected to an intracerebral hemorrhage model showing no worsening of the volume of hemorrhage or a greater functional impairment compared to the control saline group. These results led to a randomized, double-blind phase IIb clinical trial with stroke patients. A total of 50 patients were randomized to one of two study arms: the control group (placebo) and the experimental group [20 mg of riboflavin (vitamin B2 Streuli®)]. Decrease in glutamate concentrations was significantly greater ($p < 0.029$) in the treated group. Comparative analysis of the percentage improvement of the NIHSS score at discharge was slightly higher in the riboflavin-treated group compared to the placebo group (33.7 ± 43.7 vs. $48.9 \pm 42.4\%$, $p = 0.050$). This translational study represents the first human demonstration of the efficacy of blood glutamate grabbers in the treatment of patients with stroke, paving the way for the development of a promising novel protective therapy (da Silva-Candal et al. 2018).

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Chapter 2
Definitions and Concepts of Stress

Luana M. Manosso, Claudia R. Gasparini, Gislaine Z. Réus, and Zoran M. Pavlovic

Abstract Stress is a complex phenomenon that silently rises and contributes to mental health disorders and chronic health conditions, decreasing work productivity, reducing our quality of life, and increasing our medical expenditures exponentially. Although a certain amount of stress is positive and beneficial for performance, such as "eustress," chronic stress experienced for an extended time overwhelms the body's coping mechanisms. We begin our chapter by briefly mentioning historical milestones related to stress research, followed by the definitions of stress. We then discuss the most recent epidemiological data related to stress prevalence and incidence, followed by a short description of the different types of stress across the lifespan. The following sections are dedicated to Burnout Syndrome, Stress-induced Exhaustion Disorder, and other types of stress-related experiences typical for our modern societies, such as Financial Stress and Stress due to Mental Illness Stigmatization. Finally, we conclude our chapter with the latest information on Caregiver Stress and Secondary Traumatic Stress.

Keywords Acute stress · Chronic stress · Stress modalities · Eustress · Burnout · Financial stress

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_2

2.1 History of Stress

Stress has received increased attention and recognition from the medical community due to its pervasive effects on the health of the human population. Although a universal definition of stress does not exist, the word "*stress*" has made its way into many disciplines ranging from physiology to medicine, chemistry, endocrinology, neurosciences, epidemiology, psychiatry, epigenetics, and psychology, reflecting the multidimensionality and composite nature of the concept (Le Moal 2007; Robinson 2018). For instance, possible meanings include "*reaction*" (physiology), "*negatively perceived factor or situation*" (psychology), or "*environmental factors affecting the cell or organ or body*" (biology) (Bienertova-Vasku et al. 2020). We will then discuss the contributions of Hans Selye, Claude Bernard, Walter Cannon, Sir William Osler, Yerkes and Dodson, Richard Lazarus, and Bruce S McEwen to the modern concept of stress that we know today.

Hans Selye (1907–1982) was an Austrian-born Hungarian scientist who trained as a medical doctor and is cited as the "*father of stress*" because he conducted experiments in lab rats and patients whereby he demonstrated the existence of biological stress, a nonspecific response of an organism to stressors (Robinson 2018). Selye was the first to introduce the term "*stress*" into the medical lexicon, a word he borrowed from physics where the original use of the term was to refer to the force that produces strain on a certain composite material (i.e., bending a piece of metal until it snaps, occurs because of the force, or stress, exerted on it) (Tan and Yip 2018).

Selye's experiments in rats led him to propose his theory of general adaptive syndrome (GAS) in 1936, known today as "*stress response*," which follows three stages: alarm reaction, resistance, and exhaustion. In the first stage, the body's initial symptoms due to stress are experienced; in the second stage, the body produces a response to adapt to the stressor. In the final stage, exhaustion and death ensue because long-term adaptation to the stressor cannot be sustained (Selyes 1956). Moreover, Selye proposed that stress plays a role in every disease and that inability to cope or adapt to stressors can produce "*diseases of adaptation*," including gastric ulcers, high blood pressure, and heart attacks (Godoy et al. 2018). Selye's key message was that the prolonged effect of stress alters the internal equilibrium of physiological systems and leads to pathology (Le Moal 2007). In 1974, Selye expanded his theory by categorizing stress into four distinct subgroups based on the type of stressor and stress manifestation: good stress (*eustress*), bad stress (*distress*), over-stress (*hyperstress*), and under-stress (*hypostress*) (Selye 1974). He coined the term "*eustress*" from the Greek word root "*eu*-" which means "good" (as in "*euphoria*"), while the term "*distress*" stems from the Latin, "*dis*-" (as in "*dissonance*" or "*disagreement*"). Furthermore, eustress is a state manifested by feelings of excitement, fulfillment, meaning, satisfaction, and well-being and a perception of a stressor as positive and challenging. In contrast, distress is associated with a sense of suffering and decreased quality of life (Selye 1975; Le Fevre et al. 2006).

Selye is also acknowledged for his contributions to elucidating the hypothalamic-pituitary-adrenal (HPA) axis and its role in chronic stress. The HPA axis is pivotal to understanding the neurobiology of the stress response and the elaborate communication network that orchestrates the stress response, including hormones, neurotransmitters, chemicals associated with the immune system, and other molecular signaling mechanisms (Kumar et al. 2013).

The French physiologist Claude Bernard (1813–1878) is credited with developing the theory of the *"milieu intérieur,"* translated as "*the internal environment*" (Rom and Reznick 2015). Among Bernard's several contributions to medicine, his approach on how organisms maintain internal stability, i.e., "*the self-regulation of vital processes*," is considered significant to the evolution of stress research (Bernard 1872).

Walter Cannon (1871–1945), nearly 50 years later, expanded on Bernard's work and coined the term "*homeostasis.*" Cannon proposed two ways to maintain homeostasis: through the sense organs and the negative feedback of the autonomic nervous system. These discoveries laid the foundation for what has come to be understood as homeostatic mechanisms, a cornerstone of stress research (Cannon 1932). Moreover, Walter Cannon introduced specific psychological aspects of stress by formulating the "*fight or flight*" model of the stress response (Cannon 1932).

Sir William Osler (1849–1919), a Canadian doctor, is recognized for his patient-centered approach to medicine and the use of algorithmic analysis of symptoms to diagnose the disease and a method to generate symptoms through testing (Robinson 2018; John 2013).

Yerkes and Dodson are known for developing the "*Yerkes–Dodson Law*", which describes that a certain level of arousal is necessary for optimal performance. The law follows the inverted-U shape, indicating that either insufficient or excessive arousal leads to poor performance (Robinson 2018).

In 1984, Lazarus and Folkman explained stress by way of the "*transactional theory of stress and coping*" (TTSC), which they described as "*a product of a transaction between a person (including cognitive, physiological, affective, psychological, and neurological functions) and his or hers complex environment*" (Walinga 2014).

The most prominent contemporary researcher on stress neurobiology was Bruce S. McEwen (1938 - 2020), an American neuroendocrinologist whose research activity focused on studying mechanisms underlying individual responses to stress. He coined the term "*allostatic load*," which refers to "*the wear and tear on the body*" induced by stress exposure in vulnerable subjects, as opposed to "*allostasis*," which instead defines the adaptive processes that maintain homeostasis.

2.2 Definitions of Stress

Stress is typically defined as a condition where physical, mental, or emotional strain or inner tension causes restlessness, worry, and lack of sleep (Wiegner et al. 2015). Moreover, stress is considered a type of psychological pain. It proves beneficial in

the short term and small quantities, as it can improve performance and plays a significant role in motivation, adaptation, and reaction to environmental stimuli (Dhabhar 2018).

Animals adapt physiologically and psychologically to stress by turning on a wide array of cognitive, affective, behavioral, and physiological responses collectively referred to as the "*stress response*" (Guilliams 2015). The psychological aspects of stress response include heightened awareness, enhanced cognitive performance, and euphoric mood states (Guilliams 2015), which could only increase to a certain extent as according to the Yerkes–Dodson law, any further rise in stressor intensity or stress-induced activities would have detrimental effects resulting in animal exhaustion (Yerkes and Dodson 1908). The physiological adaptations manifest through increased sympathetic nervous system activation, accompanied by an enhanced release of stress hormones cortisol and adrenaline, associated with the "*fight or flight*" response (Guilliams 2015). For example, in a gazelle being chased by a tiger, the fight or flight response is an automatic reaction that prepares the gazelle to fight or flee from the tiger, which is a stressful event. The fight or flight response evolved to deal with mainly immediate threats (Sapolsky 1994). This "*adaptive stress response*" is regulated by neuronal and endocrine systems known as the HPA axis (Guilliams 2015), which is responsible for reestablishing homeostasis via the secretion of glucocorticoids, steroid hormones produced by the adrenal glands (Boudreau et al. 2011).

The stress response can also be activated without the presence of actual threat through anticipation of physical or psychological insults, due to, for example, "*negative future fiction*," which occurs when one worries about worst-case scenarios and crafts up obstacles about events one anticipate may happen yet did not happen (Sapolsky 1994). Unfortunately, or instead, fortunately, the stress response did not evolve to accommodate the cognitive worrying mind, which creates stress by worrying about "*what if I get chased by a tiger tomorrow?*"

During stressful events, the adrenal glands release adrenaline and cortisol, speed up the heart rate, slow digestion, and shunt blood flow to major muscle groups, giving the body a burst of energy and strength to either stay and fight the predator or flee it (Guilliams 2015). For the body to turn on this emergency response, it needs to shut down other activities such as feeding, digestion, growth, reproduction, and immunity. This adaptive stress response is helpful in the short term to overcome the stressful event; however, if it is constantly switched on and cortisol is continuously high, this state of repeated stress becomes known as chronic stress (Lupien et al. 2018).

Chronic stress can lead to long-term changes in HPA activity, including persistent elevations in basal glucocorticoids, abnormal responses to subsequent stressors, and impaired HPA axis feedback regulation (Herman et al. 2016). The psychological effects of reviewing the past and rehearsing future events decrease memory performance and raise cortisol levels, resulting in morphological brain changes such as reduced hippocampal (HIPP) volume (Kim et al. 2015). Although the fight or flight response is essential to survival, when it is continuously present, it results in an increased risk of developing specific neuropsychiatric disorders, causing remodeling

of brain structures and impairing immune, digestive, cardiovascular, sleep, and reproductive system functions (Musazzi et al. 2011; Tsigos et al. 2020).

Chronic stress causes the body to remain in a constant state of alertness, despite being in no danger, thus depleting the body and mind of energy and leading to a loss of motivation while increasing the likelihood of physical disorders such as cardiovascular diseases, high blood pressure, and diabetes (Seiler et al. 2019). In addition, acute and chronic stress can also accelerate aging, disease occurrence and lead to early mortality. Chronic stress can occur at any point in life (i.e., prenatal, early, and late-life) and be induced by various circumstances (i.e., psychosocial, social isolation, social-defeat, environmental, etc.) (Sheth et al. 2017). Moreover, it is considered the "*black plague*" of modern human society, with as many as 90% of physicians' visits occurring due to stress-related health complaints (Nerurkar et al. 2013). In general, stress can be triggered by a range of endogenous and extrinsic factors, usually following the "*NUTS*" mnemonic (Hogan 2013).

NUTS:

- **N**ovelty: new situation
- **U**npredictability: a situation you had no way of knowing would occur
- **T**hreat to the ego: a situation that makes you feel your competence has been questioned
- **S**ense of Control: a situation that gives you the feeling to have little or no control

2.3 Stress Epidemiology

Our review of recent publications about the epidemiology of stress-related disorders showed the preponderance of studies on posttraumatic stress disorder (PTSD) (Gradus 2017). Studies in the USA reported its lifetime prevalence of about 6.8–7.8% (Kessler et al. 1995, 2005). In other countries, such as Germany (Perkonigg et al. 2000) and Australia (Creamer et al. 2001), the prevalence of lifetime PTSD was estimated to be lower (1.3% and 1.33%, respectively). Newly available data from the World Health Organization (WHO) on the prevalence of mental disorders suggest a higher prevalence of PTSD. The WHO estimated that age-standardized prevalence for PTSD was 15.3% (9.9–23.5%) (Charlson et al. 2019). PTSD is more common among women than men in the general population (Gradus 2017). Along the same lines, a meta-analysis investigating the prevalence of PTSD in women during pregnancy and after birth showed that the prevalence of PTSD was 3.3% and 4.0%, respectively (Yildiz et al. 2017).

Another group showing high levels of stress is healthcare professionals. In a cohort of Australian nurses, the prevalence rate of stress was 41.2%, with job dissatisfaction as the most significant predictor of a higher risk for developing symptoms of depression and stress (Maharaj et al. 2019). A multicenter survey of anesthesiologists and nurses that work in intensive care units demonstrated that both groups experienced medium stress levels. Interestingly, nurses showed significantly

higher stress levels than physicians, with women showing higher levels of stress than men (Kwiatosz-Muc et al. 2018). Finally, a systematic review and meta-analysis of Brazilian medical students reported that the prevalence of stress was 49.9% (Pacheco et al. 2017).

Several recent epidemiological studies report the continuous increase in stress-related psychological symptoms in the last two decades. For instance, a nationally representative survey from the USA has shown that between 2008 and 2017, the proportion of young adults (18–25 years old) that experienced severe psychological distress in the last 30 days increased by 71% (Twenge et al. 2019). In 2020, due to the COVID-19 crisis, psychological distress was reported more frequently than before the pandemic. A recent meta-analysis demonstrated the prevalence of stress symptoms in 29.6% of survey participants. The same study also noted anxiety symptoms in 31.9% and depressive symptoms in 33.7% of the analyzed sample (Salari et al. 2020), probably induced or aggravated by highly stressful circumstances due to the ongoing pandemic. Finally, a population-based COVID-19 study with individuals living in the USA found that younger age, female gender, and caregiver status increased risk for stressor exposure and a degree of an event's stressfulness (Park et al. 2020).

2.4 Eustress

According to Hans Selye, eustress or "*good stress*" refers to a psychological response to a stressor that is interpreted as having positive implications on one's well-being (Selye 1974). Thus, eustress is beneficial for enhancing motivation and performance. Still, once the peak performance level is achieved, it starts declining and is associated with an individual's distress (Benson and Allen 1980).

Positive stress at work is the psychological state with a mindful focus on challenges present in the organizational environment (Quick et al. 2013). Experiences of eustress lead to increased concentration and a form of arousal that enhances attention on the task (Hargrove et al. 2013). Eustress is a positive psychological response to various interpersonal, physical, job role demands, and workplace policies. It increases work performance, overall health, and subjective well-being (Hargrove et al. 2013; Simmons and Nelson 2001; Nelson and Cooper 2007).

The brain is the central organ responsible for regulating stress response and adaptation to the stressor. That is why, as soon as it perceives a stimulus as stressful, physiological and behavioral stress responses are triggered, resulting in the HPA axis activation (Esch and Stefano 2010). Once activated, it starts releasing corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary (AP) to secrete adrenocorticotropic hormone (ACTH), which in turn induces a release of cortisol from the adrenal cortex into the bloodstream (Sapolsky et al. 2000). The HPA axis is vital for normal physiological functions and the regulation of other systems. For example, cortisol is essential to mobilizing energy resources to provide "*fuel*" for the body. It also inhibits immune system functioning and has a

permissive effect, allowing other physiological systems to function effectively. Moreover, the HPA axis activation is associated with critical cognitive and affective processes and is thought to significantly impact health and disease occurrence and progression (Dickerson and Kemeny 2004).

Interestingly, in a clinical experiment, Akinola et al. demonstrated that cortisol increase was positively related to the performance of participants who were told to view anxiety as beneficial for negotiation performance (appraisal group). In contrast, cortisol increase was negatively associated with negotiation performance of participants given no instructions on appraising their anxiety (control group) (Akinola et al. 2016).

Eustress is also closely related to the concept of flow (Csíkszentmihályi 2008). During the "*state of flow*" time suspends, individuals lose themselves in the activity while perceiving great control over work tasks, accompanied by a deep sense of enjoyment and creativity associated with peak performance (Hargrove et al. 2013). After evaluating brain scans of individuals experiencing flow, Daniel Goleman, a world-renowned psychologist, noted intensive activation of the left prefrontal area, which contains the circuitry that lights up when positive emotions are active. He also suggested that brain chemistry changes such as increased dopamine levels might also accompany flow states, benefiting both mood and performance (Goleman 2011).

Stress produces a string of phenotypic trajectories, depending on environmental stimuli intensity, type, and duration. Moreover, depending on the specific evaluation time point(s), the reactions to stressors might manifest either as eustress or distress. An example of this phenomenon is a reaction to a common stressor in academic life, for instance, examination or homework deadline. The situation usually develops as follows: upon hearing about a new tight deadline, many students experience discomfort or even anxiety and decrease in day-day performance (distress); close to the deadline, they frequently report a sense of increased motivation and productivity (eustress), and following the deadline, they are feeling exhausted, and experience decline in everyday performance (distress). Importantly, defining eustress simply by peak performance may be too simplistic, as psychostimulants such as methamphetamine or cocaine can also have similar effects but, in the long run, are very detrimental for psychological and physical health (Bienertova-Vasku et al. 2020). Finally, most neuroscientists prefer to consider eustress as an adaptation process, labeling eustress a type of stress that increases an organism's adaptive capacity to a specific life situation (Kupriyanov and Zhdanov 2014).

2.5 Early Life Stress

Early life stress is any form of severe trauma such as physical, emotional, sexual abuse or negligence, parental loss, or exposure to natural disaster experienced during prenatal life, first months of life, early and late childhood, or adolescence (Enoch 2011; Allen and Dwivedi 2020; Agorastos et al. 2019). It predisposes individuals to develop various psychiatric disorders, mainly anxiety disorders and major

depressive disorder (MDD) (Juruena et al. 2020; Łosiak et al. 2019). Along the same lines, a meta-analysis by Nanni et al. reported that childhood maltreatment was associated with an elevated risk of developing recurrent and persistent depressive episodes, including a lack of response to antidepressant treatment (Nanni et al. 2012). Early life stress is also a predictor of co-occurring alcohol use disorder and PTSD (Lee et al. 2018). The severity of childhood maltreatment is positively correlated with inferior quality of life in adulthood (Enoch 2011).

The severity of childhood trauma experience also contributes to increased HPA axis activity (Nikkheslat et al. 2020). Males with depression and early life stress exhibit enhanced inflammatory responsiveness to psychosocial stress, indicating a possible link between depression, early life stress, and adverse health outcomes associated with systematic inflammation (Pace et al. 2006).

Animal studies complement human cross-sectional and longitudinal studies that are helping us to understand the effects of early life stress (Marco et al. 2015). For instance, maternal deprivation or maternal separation protocols are widely used to study the consequences of early life stress (Réus et al. 2011; El Khoury et al. 2006; Vetulani 2013; Lee et al. 2007). Like humans, maternal deprivation in rodents induces depressive-like behavior, anhedonia, anxiety, and cognitive impairment (Marco et al. 2013, 2015; Réus et al. 2011; Ahmad et al. 2018). Multiple studies using maternal deprivation protocols also showed increased inflammation (systemic and neuroinflammation) and oxidative stress (Réus et al. 2015, 2017), co-occurring with changes in brain neurotrophins (Réus et al. 2011), various neurotransmitters (Marco et al. 2015; Lee et al. 2007), and gut-microbiota (Rincel et al. 2019). Maternal deprivation effects vary according to the developmental stages and sex. For example, it was noted that depressive behaviors occur typically in adulthood, while inflammation and microglial activation predominate in early life, including infancy and adolescence (Giridharan et al. 2019; Réus et al. 2019).

Numerous studies also indicated that epigenetic mechanisms might be involved in the consequences of early life stress (Torres-Berrío et al. 2019; Heim and Binder 2012; Li et al. 2020a; Murgatroyd et al. 2009). A review by Allen and Dwivedi showed that microRNAs could also play a role in the maladaptive processes associated with early life stress, both at adolescent and adult age (Allen and Dwivedi 2020).

Finally, not all children exposed to maltreatment develop psychopathology, indicating that resilience and other mediating factors such as the gene–environment interactions also play a significant role in modulating individual stress responses (Enoch 2011).

2.6 Acute Stress

Acute stress is a state in which homeostasis is threatened by immediate adverse extrinsic or intrinsic forces (stressors) and is associated with a physiological response designed to meet the instant challenge (Esch and Stefano 2010).

In response to stress, the cells of the paraventricular nucleus of the hypothalamus secrete CRH, which acts on the AP, activating ACTH release. The ACTH is then secreted into the bloodstream, stimulating the release of glucocorticoids from the adrenal gland cortex.

Glucocorticoids play a principal role in energy metabolism, growth processes, immune and brain functions underlying behavioral adaptation. They exercise a negative feedback loop at the hypothalamus and pituitary gland levels to shut down CRF and ACTH (Carrasco and Van De Kar 2003; Xiong and Zhang 2012). Although short-term increases in cortisol can be adaptive, preparing the body to respond to a stressor, prolonged cortisol elevation can have harmful effects on the brain, behavior, and cognition (see the subsection on "*chronic stress*") (Dickerson and Kemeny 2004; Lupien et al. 2009).

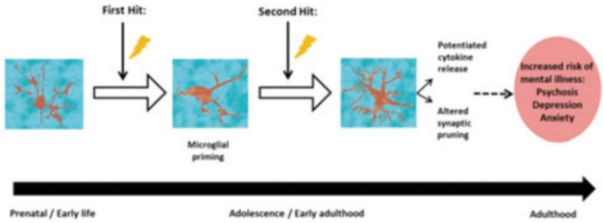
CRH also acts as a neurotransmitter or neuromodulator in the amygdala (AMG), dorsal raphe nucleus (DRN), HIPP, and locus coeruleus (LC), integrating the brain responses to stress. For example, CRH increases noradrenergic neurotransmission in the LC, followed by decreased neurovegetative functions, such as eating and sleeping (Carrasco and Van De Kar 2003).

Specific physiological changes associated with the response to acute stress generate the fight or flight response, which includes: (a) mobilization of energy to maintain brain perfusion rates and use of glucose; (b) increased brain and muscle function; (c) enhanced attention on the perceived threat; (d) increased cardiovascular output and respiration, and redistribution of blood flow, increasing the delivery of nutritious substances and energy to the brain and muscles; (e) modulation of immune function; (f) inhibition of reproductive physiology and sexual behavior; and (g) decreased feeding and appetite (Carrasco and Van De Kar 2003; McCarty 2016).

When acute stress is very intense and traumatic, for example, in threatened death, serious injury, or sexual violation, acute stress disorder (ASD) may develop, causing clinically significant distress or impairment in social, occupational, or other important areas of functioning with a duration of 3 days to 1 month following exposure to the traumatic event (American Psychiatric Association 2013).

2.7 Chronic Stress

Chronic stressors usually permeate people's life, and unlike acute stress, the person exposed to chronic stressful conditions either does not know whether or when the challenge will end (Segerstrom and Miller 2004). Chronic stress can take many different forms and is often characterized by variability in duration and intensity (Rohleder 2019). Acute stress is considered primarily as a form of adaptation to ongoing circumstances. Usually, it does not have long-term consequences as chronic stress, which is heavily implicated in causing ill-health conditions, including major psychiatric disorders (Menard et al. 2017), poor overall mental health (Cattaneo and Riva 2016), gastrointestinal disorders (Alonso et al. 2008), cardiovascular disease (Yao et al. 2019), and cancer (Dai et al. 2020).



The 'two-hit' hypothesis: exposure to prenatal/early-life stress (*lightning bolt*) may act to prime microglia within the CNS so that a subsequent challenge later in life, either in adolescence or adulthood invokes a potentiated microglial response, leading to an increased risk of developing a mental illness

Fig. 2.1 The figure presents a simplified mechanism explaining how early exposure to stressors is priming microglia, leading to neuroinflammation and increased risk for developing stress-related disorders (from Calcia et al. 2016)

Chronic stress results in hypersecretion of adrenal glucocorticoids and sustained activation of the central and peripheral sympathetic systems. During the state of chronic stress, the negative feedback loop through which cortisol regulates the ongoing release of CRF breaks down, and despite the glucocorticoid receptor downregulation, the release of CRF continues without cessation. Finally, the rise in the plasma cortisol concentration in chronic stress is further enhanced by releasing arginine-vasopressin (AVP) from the hypothalamus (Leonard 2005; Aguilera 1994).

Another consequence of chronic stress is a dysregulation of the immune system as both chronic external stressors and stress hormones act to up-regulate the expression of stress-related pro-inflammatory genes, thereby increasing the release of pro-inflammatory cells and the production of pro-inflammatory cytokines, such as interleukin-1 (IL-1) IL-6, and tumor necrosis factor α (TNF-α) (Cohen et al. 2012). Besides glucocorticoids, the sympathetic nervous system and its main neurotransmitter norepinephrine and neuropeptide Y are also involved in regulating the immune function and inflammatory processes during the stress response (Dai et al. 2020; Liu et al. 2017a).

In addition to peripheral inflammation, inflammation in the brain, namely "*neuroinflammation*", has also been found in individuals experiencing chronic stress. Peripheral cytokines can penetrate the central nervous system (CNS) through the circumventricular organs (CVOs), brain structures located around the third and fourth ventricles, characterized by the lack of a blood-brain barrier, increasing microglial activation (Munhoz et al. 2008; Calcia et al. 2016; Miller et al. 2009; Kaufmann et al. 2017) (see Fig. 2.1).

Stressful events are often followed by alterations in oxidative/nitrosative cellular pathways in the brain in response to inflammatory mediators (Munhoz et al. 2008). These changes can trigger neurodegenerative diseases, major psychiatric disorders, and other adverse health conditions, such as cerebral ischemia (Wang and Michaelis 2010; Salim 2017). Moreover, central, peripheral inflammation and oxidative stress can impact the functioning of the brain and other body organs, thus increasing the risk for the development of multiple systemic illnesses (Yao et al. 2019; Ambrósio et al. 2018; Carrier 2017; Cobley et al. 2018).

Depending on its intensity or duration, stress can also trigger molecular (e.g., decreased neurotrophic factors and increased glutamate excitotoxicity), cellular (e.g., reduced neurogenesis and synaptic plasticity), or tissue (e.g., decreased HIPP volume and neuronal function) alterations that can predispose an individual to the development of psychiatric and neurological disorders (Duric and Duman 2013). Moreover, stress can induce cognitive, emotional processing, and behavioral deficits by affecting glutamate release, transmission, and metabolism in cortical and limbic brain areas (Popoli et al. 2012). It can also alter lipid metabolism and glycemic control and affect diverse hormonal systems, impacting mental and physical health through several mechanisms simultaneously (Yao et al. 2019; Scott et al. 2012).

2.8 Chronic Stress and Gender Differences

The mechanisms to cope with stressors differ between males and females (Bale and Epperson 2015). Differences in gonadal steroids are primarily driving sex-specific responses to physical or psychological threats (Oyola and Handa 2017)

As reviewed by Oyola and Handa (Oyola and Handa 2017), significant sex differences related to the HPA axis reactivity are that: (a) females reportedly have more prominent paraventricular nucleus AVP neurons than males; (b) the release of CRH triggers a more pronounced ACTH response in female than in male rats; (c) glucocorticoid increases following a stressor are higher and remain elevated for a longer time in female rats; (d) the corticosteroid-binding globulin (CBG—a protein that controls the glucocorticoid bioavailability in the plasma) is higher in female than in male rats, and (e) the estrogens regulate negative feedback at multiple sites. Moreover, the fluctuations in gonadal hormones in females have also been shown to modulate how females react to stress (Oyola and Handa 2017).

Furthermore, sex differences in the neuropeptide Y system seem to be involved in the stress response modulation. As recently reviewed by Nahvi et al. the female gender has lower neuropeptide Y levels and fewer neuropeptide Y-expressing cells than males, making them more susceptible to stressors (Nahvi and Sabban 2020). Several clinical studies related neuropeptide Y with individual resilience to stress, with its lower levels associated more often with the presence of stress-related disorders (Sah et al. 2009, 2014). In their set of preclinical studies, Monch et al. reported significant effects of chronic stress on the medial prefrontal cortex (mPFC) and HIPP neuronal architecture in adult males, compared to a few neurobiological changes found in adult female rodents (Moench et al. 2019). Finally, the sex-specific neuroimmune effects of stress also significantly contribute to stress response and resilience (Bekhbat and Neigh 2018).

A clinical study analyzing electroencephalography (EEG—reflects correlated synaptic activity caused by post-synaptic potentials of cortical neurons) and functional magnetic resonance imaging (fMRI—uses changes in blood flow as indices of activity in the brain) revealed sex differences between individual subject responses. Women mainly showed faster frontal cortical EEG responses to negative stimuli.

Moreover, LC was more activated in women than in men during stressful events (Bangasser et al. 2018). An additional difference between the two genders frequently reported by neuroscientists is the men's typical fight or flight and women's "*tend and befriend*" behavioral response following exposure to the same stressful situations (Verma et al. 2012; McEwen 2017).

Similarly, in animal experiments, male and female rodents appear to be differentially affected by the chronic mild stress model, depending on their behavioral, physiological, and neurobiological indices being measured (Franceschelli et al. 2014). For example, the chronic stress paradigm impaired learning and memory processes more in males than female mice. In contrast, chronic stress increased depressive-like behavior more in females than males (Franceschelli et al. 2014; Luine et al. 2017; Sachs et al. 2014). The relationship between levels of gonadal hormones and psychological states is further evidenced by the fact that more than half of women with MDD experienced increased severity of depressive symptoms during the premenstrual phase of their regular menstrual cycle (Altemus et al. 2014).

Finally, elevated stress hormones can also negatively impact the reproductive neuroendocrine axis by affecting the levels of circulating gonadal hormones, thus compromising the overall reproductive function (Rivier and Rivest 1991; Whirledge and Cidlowski 2010).

2.9 Chronic Stress in Elderly

Brain regions that undergo the most rapid decline due to aging are also highly susceptible to the effects of stress hormones. The AMG, FC, and HIPP seem to be the most affected ones (Lupien et al. 2009). Liu et al. reported a positive correlation between the intensity of perceived stress and the severity of depression in the elderly, so the more significant was the perception of stress, the more severe were the depressive symptoms (Liu et al. 2017b). Moreover, a cross-sectional study with ninety elderly stroke survivors showed that a higher perception of stress was positively correlated with a degree of patient's dependence on others in daily functioning (dos Santos et al. 2015). Mills et al. reported that high levels of chronic stress were associated with increased plasma norepinephrine concentration in spousal caregivers of Alzheimer's disease (AD) patients (Mills et al. 1997). Another study in elderly caregivers with chronic pain conditions found a positive association between the severity of perceived stress symptoms and the number of different pain relief medications used (Terassi et al. 2020).

Stress can also negatively affect cognitive health in the elderly. For instance, Sroykham and Wongsawat showed that delayed recall was positively correlated with salivary cortisol levels (Sroykham and Wongsawat 2019). Along the same lines, higher plasma cortisol levels in the elderly with AD were associated with a more rapid increase in symptoms and more severe deterioration in performance on neuropsychological tests assessing temporal lobe functions (Csernansky et al. 2006). Similarly, Ennis et al. reported that cortisol dysregulation was a significant predictor

of risk for AD development occurring on average 2.9 years before AD onset (Ennis et al. 2017). Premature aging and dementia in individuals with PTSD are frequently associated with chronic re-experiencing of traumatic stress symptoms (Jakel 2018).

Due to several recent global economic crises, insufficient income, and increased longevity, the number of elderly workers is steadily growing. It was noted that older employees are more susceptible to adverse effects of chronic occupational stress than their middle-aged colleagues, mainly due to several concomitant chronic diseases (Ha and Kim 2019).

A cross-sectional study by Kumar et al. of elderly living in rural areas of Thailand showed that chronic stress was significantly associated with comorbid alcohol use disorder and a higher incidence of chronic illnesses (Kumar et al. 2020). Another study conducted among older women in four U.S. communities, followed from 1999 to 2007, reported that the women experiencing high-stress levels had greater mortality risk than those in the low-stress group but only during the first 3 years of follow-up (Fredman et al. 2010). Interestingly, in the study of elderly aged 65 years or older, when looking at the association between social connectedness, such as helping others, stressful events, and mortality risk, Poulin et al. observed a positive correlation between the number of stressful events and mortality risk, only in participants who did not help other people. No such association was found in those compassionate to others who lived longer and reported better overall health status. Based on these findings, the researchers strongly suggested that helping others might serve as a stress buffer with additional psychosocial benefits that promote good health and increase overall well-being and longevity (Poulin et al. 2013).

2.10 Chronic Stress and Aging

During adulthood, the brain regions that undergo the most rapid decline are at the same time the most susceptible to the effects of stress hormones. Accumulation of stress increases allostatic load and might result in a specific stress-related disorder or presents as nonspecific chronic stress symptomatology (Lupien et al. 2009). A longitudinal study by Lupien et al. found that participants with chronically elevated cortisol levels had morphological changes in the brain structures, such as decreased HIPP (Lupien et al. 1998). A preclinical study showed that chronic stress alters the expression levels of longevity-related genes in the rat HIPP, suggesting that chronic stress might serve as the accelerator of HIPP biological aging (Sánchez-Hidalgo et al. 2016). Aging-related changes in rodents mimic hallmarks of chronic stress effects observed in the human population, manifested as immune system dysregulation, cognitive decline, and decreased synaptic plasticity, occurring primarily in stress-susceptible regions (Barrientos et al. 2012; Novais et al. 2017).

Aging is also associated with an enhanced HPA axis responsiveness manifested by continuously elevated cortisol levels (Raskind et al. 1994; Woods et al. 2006; Moffat et al. 2020). In the elderly, a significant positive correlation between

low-grade inflammation (evaluated by C-reactive protein levels) and urinary cortisol level was observed (Martocchia et al. 2020). This type of low-grade inflammation called "*inflamm-aging*" is a hallmark of the aging process and increases the risk for multimorbidity, physical and cognitive disability, and frailty (Bektas et al. 2018). Inflamm-aging derives from several interdependent factors: mitochondrial dysfunction, dysregulation of the immune system, hormonal changes, epigenetic modifications, and other abnormalities (Fougère et al. 2017; Franceschi et al. 2007). Aging is also accompanied by progressive immune system functional deficiencies, called "*immunosenescence*," which increases the risk for infection and compromises wound healing (Heffner 2011). Moreover, the immunological function is affected by dehydroepiandrosterone (DHEA), another biomarker of chronic stress secreted by the adrenal cortex in response to CRH and ACTH stimulation with opposing effects on the immune system when compared to cortisol. With aging, DHEA secretion in response to ACTH stimulation decreases while the cortisol/DHEA ratio increases (Heffner 2011).

Preclinical data have also identified the negative impact of aging-induced gonadal hormone changes on genes involved in a stress response modulation. The consequences of this interaction mainly manifest as alterations in morphology and neurochemistry within specific brain regions, critical for executive functions, learning, memory, and stress regulation (Bale and Epperson 2015).

2.11 Financial Stress

From a financial institution perspective, "*financial stress*" (FS) is always present to a degree somewhere in the financial system, and according to Illing and Liu, is intensified during, for instance, an economic crisis when either expected financial loss, an increased risk (a higher probability of loss) or uncertainty (reduced confidence about the possibility of loss) rises (Illing and Liu 2006). On a personal level, FS is usually considered as the inability to meet one's financial obligations, causing psychological or emotional reactions such as worry, or a sense of scarcity, accompanied by the physiological aspects of the stress response (Northern et al. 2010). For example, a research study by Andrews and Wilding showed that financial difficulties could increase anxiety, depression and affect academic performance in British students (Andrews and Wilding 2004). A similar study indicated that FS is widespread among students, with 71% of the participants feeling stressed due to personal economic issues (Heckman et al. 2014). In older adults, financial strain is also very often associated with depression (Krause 1987). In contrast, people with higher financial self-efficacy (i.e., those who manage their money well) and greater optimism about their financial future are significantly less likely to report experiencing FS (Heckman et al. 2014).

Research has also investigated the impact of FS on people with certain illnesses. For example, a study of arthritis patients found that financial stressors significantly affected the physical and emotional well-being of the participants (Skinner et al.

2004). Similarly, FS due to a recent diagnosis of cancer is highly correlated with increased risk of adverse psychological outcomes like depression and anxiety (measured by Depression Anxiety Stress Scales-21) (Sharp et al. 2013) and low health-related quality of life (measured by QLQ-C30—a quality of life questionnaire used by the European Organization for Research and Treatment of Cancer) (Sharp et al. 2018). Moreover, following the admission to the intensive care unit due to critical illness prevalence of FS was high, especially in females, and was associated with anxiety, depression, and decreased overall mental health (Khandelwal et al. 2018). A study by Khandelwal et al. also suggested a complex interplay between critical illness, the need for high-intensity care, family situation, and patient's FS (Khandelwal et al. 2020).

A recent population-based study in the USA observed that during the COVID-19 pandemic, individuals who reported financial strain appear to be at increased risk for stressor exposure. Of the stressors experienced, the most stressful were those related to the loss of job security or income, risk of a loved one's illness, loss of job/education, and lack of access to information (Park et al. 2020).

2.12 Chronic Stress and Burnout

The term "*burnout*" was introduced by Freudenberger in 1974 when he observed a loss of motivation and reduced commitment among volunteers at a mental health clinic (Freudenberger 1974). However, this concept did not get too much attention until 1981 when Christina Maslach and Susan E. Jackson developed the "*Maslach Burnout Inventory*," which is still the most widely used instrument to measure this occupational phenomenon (Maslach and Jackson 1981). According to Maslach, burnout is defined as a prolonged response to chronic emotional and interpersonal stressors on the job. It is manifested by emotional exhaustion (feeling tired and powerless to provide more support to others), depersonalization (showing a disengaged, cynical, cold, and unsympathetic attitude toward colleagues), and reduced personal accomplishment (decline in feelings of competence and performance at work) (Maslach et al. 2001).

"*Burnout syndrome*" (BOS) occurs predominantly in people who work in posts that involve frequent and intense contact with people. Several studies demonstrated that BOS presents a significant problem among healthcare workers, especially nurses and physicians (Friganović et al. 2019; De La Fuente-Solana et al. 2019; Bridgeman et al. 2018; Rotenstein et al. 2018). A systematic review and meta-analysis by Rodrigues et al. also reported its high prevalence among medical residents (35.7%), which was significantly higher among surgical/emergency medicine residents than in other clinical specialties (Rodrigues et al. 2018). Although numerous adverse job characteristics could induce BOS, increased workload, low staffing levels, long shifts, and low job control are the most commonly reported (Dall'Ora et al. 2020).

Several consequences to workers' mental and physical well-being are related to BOS, including physical and psychosomatic problems. According to the recent systematic review, BOS was a significant predictor of type 2 diabetes, coronary heart disease, musculoskeletal pain, prolonged fatigue, headache, gastrointestinal issues, respiratory problems, severe injuries, and mortality below the age of 45 years. Depression, anxiety, and insomnia were the most frequently reported psychological symptoms (Salvagioni et al. 2017), while cognitive impairments have been observed in people with chronic BOS (Sandström et al. 2005). Finally, a recent meta-analysis of observational studies identified BOS as one of the most significant predictors of sickness absence (Duijts et al. 2007), while a decrease in burnout symptoms was associated with less absenteeism due to illness (Borritz et al. 2006).

2.13 Stress-Induced Exhaustion Disorder

Long-term chronic stress can lead to a state of exhaustion with the character of an illness, which is associated with changes in brain structure and function (Wallensten et al. 2019; Blix et al. 2013; Savic et al. 2018). When discussing workplace stress, some countries, like Sweden, mainly describe the occurrence of a "*stress-induced exhaustion disorder*" (SED) or "*exhaustion disorder*" (ED), which presents as mental exhaustion induced by a long-lasting period of high intensity of stress without sufficient recovery. The primary reason for including SED in the International Classification of Diseases, Tenth Revision, Swedish Version (ICD-10-SE) was to capture the symptomatology of "*clinical burnout,*" i.e., the sequelae of chronic stress without recovery (Wallensten et al. 2019). In contrast, BOS is recognized as an occupational phenomenon, not a disease.

Typical symptoms of SED are reduced mental energy, lack of endurance, and increased time needed for recovery after mental effort frequently accompanied by somatic symptoms (Wallensten et al. 2019). A clinical study with people experiencing ED showed that almost all individuals (98%) reported at least one somatic symptom, while 45% reported six symptoms or more. Nausea, flatulence, or indigestion were the most frequently reported somatic symptoms (67%), followed by headaches (65%) and dizziness (57%) (Glise et al. 2014).

Morphological changes in the brain were also observed in individuals with SED, with the frontostriatal circuits (rostral PFC, the posterior parietal cortex, and the striatum) primarily affected. The same study demonstrated that employees with high-stress levels needed to recruit additional cognitive resources to uphold task performance (Gavelin et al. 2017). This finding aligns with scientific knowledge regarding the crucial role of the striatum in working memory processing, serving as a gating mechanism that regulates the updating of working memory representations in the PFC (O'Reilly 2006). Accordingly, following stress rehabilitation (the intervention consisted of twenty-two group sessions and two individual meetings with the group therapist for 24 weeks), the striatal activity decreased due to reduced stress levels (Gavelin et al. 2017).

An MRI clinical study in patients with SED demonstrated that changes in brain regions critical for stress processing were dynamically correlated with the degree of perceived stress, highlighting a possible causal link. In addition, the morphological abnormalities were more pronounced in women, so the authors suggested that they might represent the biological substrate underlying increased female vulnerability to stress-related psychiatric disorders (Savic et al. 2018).

Biochemical alterations are also frequently noted in people with SED. For instance, several studies reported that women with SED had significantly higher plasma levels of vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) compared to healthy controls (Wallensten et al. 2016; Nowacka et al. 2015; Luger et al. 1988).

2.14 Occupational Stress

"*Occupational*" or "*work-related stress*" is determined by several factors such as lack of resources and equipment, inadequate management style, poor job design, lack of vocational and soft skills, and negative employee interactions. It most often occurs when the job demands do not match or exceed the employee's capabilities, resources, or needs, or when the knowledge or abilities of an individual worker or group are not matched or aligned with the company's expectations. Nowadays, occupational stress (OS) is generally acknowledged as a global phenomenon with immense health and economic consequences (International Labour Organization 2016).

OS is a significant health problem for both employees and organizations and can lead to BOS, illness, and increased employee turnover, presenteeism, and absenteeism (Nowrouzi et al. 2015). Moreover, workers with high levels of OS tend to have lower work engagement (Cordioli et al. 2019). High levels of OS are also associated with poor mental health outcomes (Fortes et al. 2020).

Morphological and volumetric changes in the brain are observed in people with high levels of OS. For example, a clinical study by Blix et al. reported that people experiencing symptoms of workplace stress exhibited reductions in the gray matter volumes of the ACC and dorsolateral PFC. Moreover, their caudate and putamen volumes were also reduced (Blix et al. 2013) (see Fig. 2.2). A similar study by Savic et al. showed that OS caused selective volumetric changes in subcortical regions (Savic 2015).

Occupational stress can also induce alterations in other organ systems. For instance, a systematic review by Järvelin-Pasanen (Järvelin-Pasanen et al. 2019) reported on the positive correlation between heightened occupational stress and lowered heart rate variability due to reduced parasympathetic activation (Järvelin-Pasanen et al. 2019). Furthermore, in a study by Chandola et al. employees chronically exposed to workplace stressors (arbitrarily defined as three or more significant workplace stressor exposures) were more than twice as likely to have metabolic

	Controls > Patients			Patients > Controls
	Z-level	Size (cm^3)	Coordinates	
Left middle frontal gyrus	4.3	1.9	25–11 59	
Anterior cingulate cortex (BA 32) #	3.4	0.5	−39 6 46	NONE
Right middle frontal gyrus	5.0	2.1	−2 31 13	

Cluster showing a significant group difference when using a limbic mask comprising the anterior cingulate cortex, the mPFC, the insular cortex, the hippocampus and the amygdala, using peak threshold at $p = 0.001$, FDR corrected at $p < 0.05$. The other clusters were calculated with same level of significance, but using the entire brain as search space (no a priori hypothesis).
No group differences were observed in white matter volumes and no significant clusters were detected when reversing the contrast (using the contrast: stressed patients – controls).
doi:10.1371/journal.pone.0064065.t003

Fig. 2.2 The figure presents the gray matter (GM) volumetric changes of stress-susceptible brain structures (i.e., HIPP, caudate, and putamen), showing that stressed subjects exhibited significant reductions in the GM volumes of the ACC and the dorsolateral PFC in comparison with unstressed controls (from Blix et al. 2013)

syndrome than those who did not experience substantial work-related stress (Chandola et al. 2006).

Due to the omnipresence of diverse occupational stressors in contemporary working environments, implementing evidence-based stress management practices that will prevent suffering and increase employee well-being is a must, according to Quick and Henderson, experts on workplace stress prevention (Quick and Henderson 2016).

2.15 Caregiver Stress

"*Caregiver stress*" is usually defined as the unequal exchange of assistance among people who stand in close relationships, resulting in the caregiver (Llanque et al. 2016) emotional and physical stress. A prospective cohort study conducted in older women in four U.S. communities, followed from 1999 to 2007, showed that caregivers were more stressed than non-caregivers (Fredman et al. 2010).

Caregiver (di)stress is primarily associated with the caregiving of people with chronic illnesses, like cancer (Gaugler et al. 2008; Kim et al. 2006), schizophrenia (Caqueo-Urízar et al. 2009; Stanley et al. 2017), and neurodegenerative diseases (Zwerling et al. 2016; Gilhooly et al. 2016; Krishnan et al. 2017). For instance, a study by Ferrara et al. with AD patients reported that the severity of memory problems associated with behavioral and psychological symptoms such as aggression and hallucinations was highly predictive of caregiver distress (Ferrara et al. 2008). Caregivers of children with neurodevelopmental disorders also experience significant stress, especially those taking care of children with autism spectrum disorders (ASD) (Duarte et al. 2005; Baker-Ericzén et al. 2005). For instance, parents of children with ASD experienced consistently higher stress levels during their children's early development than parents of children with non-ASD developmental concerns (DesChamps et al. 2020). As in AD informal caregivers, higher

levels of parental distress were associated with the increased severity of ASD symptomatology.

Caregiving often results in chronic stress, compromising the caregiver's physical and psychological health integrity (Schulz and Sherwood 2008). For example, a study with primary caregivers of elderly relatives with dementing illnesses showed that higher stress levels were correlated with poorer self-reported health, increased unhealthy behaviors, and greater use of health care services (Son et al. 2007). Along the same lines, a recent review demonstrated that worsening of informal caregiver's health led to the early nursing home placement of demented family members (Etters et al. 2008).

Caregivers of people with dementia have also shown increased levels of anxiety (Cooper et al. 2007) and depressive symptoms (Covinsky et al. 2003; Bin et al. 2015). A high prevalence of depression was also observed among caregivers of cancer patients (Mei et al. 2018).

Chronic stress and perceived loneliness of dementia caregivers also disrupt their neuroendocrine and neuroimmunological systems (Kovaleva et al. 2018), inducing a chronic systemic low-grade inflammation and increasing pro-inflammatory cytokine levels, ultimately leading to higher illness risk (Von Känel et al. 2006; Gouin et al. 2012; Mausbach et al. 2011; Kiecolt-Glaser et al. 2011). Along the same lines, caregivers experiencing increased stress have a higher risk for cardiovascular disease (Haley et al. 2010; Roepke et al. 2012; Mausbach et al. 2010) and higher mortality rates (Schulz and Beach 1999).

2.16 Secondary Exposure to Trauma (Secondary Traumatic Stress and Vicarious Traumatization)

McCann and Pearlman were the first to introduce the concept of "*vicarious traumatization*" (VT) in the nineties, which they noted in individuals who regularly worked with victims of traumatic events, manifested primarily by significant changes in a professional's core beliefs about themselves, others, and the world in general, due to their chronic empathic engagement (Collins and Long 2003). In addition, VT can impact a personal life, such as relationships with family and friends (Bober and Regehr 2006; Baird and Kracen 2006). Previously called "*compassion fatigue*," "*secondary traumatic stress*" (STS) refers to secondary or indirect exposure to trauma and is a set of psychological symptoms that typically mimic PTSD or ASD acquired due to exposure to individuals suffering from the effects of severe trauma. It is the consequence of engaging in an empathic relationship with an individual suffering from a traumatic experience and bearing witness to the intense or horrific experiences of that particular person's trauma.

STS and VT have been continuously reported in mental health professionals (e.g., psychiatrists, nurses, therapists), child protection workers, clergy, social workers, humanitarian aid workers, and workplace lay trauma counselors exposed chronically

to people suffering from trauma (Beck 2011; Sinclair and Hamill 2007; Shah et al. 2007; McNeillie and Rose 2020). VT symptoms typically manifest as apathy, exhaustion, irritability, cynicism, disillusionment, and altered cognitive schemas related to personal safety, trust, power, and intimacy (Isobel and Angus-Leppan 2018; Pross 2006). Interestingly, scientists studying the neurobiology of empathy discovered a group of neurons they named "*mirror*" neurons (neurons that mirror the behavior of the other person, for instance, during a conversation or socializing, as though the observer were itself acting) in the premotor cortex, the supplementary motor area, the primary somatosensory cortex, and the inferior parietal cortex, and postulated that they might be a causative factor leading to the increased occurrence of vicarious trauma among the professional groups mentioned before (Isobel and Angus-Leppan 2018). A survey involving 214 general public participants and 526 nurses (i.e., 234 front-line nurses and 292 non-front-line nurses) evaluating VT (using a mobile phone app-based questionnaire) caused by the COVID-19 pandemic showed that both groups of nurses suffered. However, surprisingly, the VT of non-front-line nurses was more severe than those of front-line nurses (Li et al. 2020b). The authors explained that this might occur due to their lack of working experience and poorer psychological coping capabilities when compared to front-line nurses. Moreover, they suggested that while the VT of front-line nurses stemmed only from empathizing with patients infected with the COVID-19, non-front-line nurses, besides feeling compassion for their patients, were also bearing the worry and sympathy for their front-line colleagues (Li et al. 2020b).

The previous history of personal and professional trauma, individual perception of being adequately trained for working with traumatized people, supervision of peer workers, the opportunity for consultation with colleagues, availability of social support, adequate self-care, sufficient leisure time, and enough engagement in other activities which are promoting psychological resiliency, are some of the factors that may affect VT's severity (Jordan 2010). For example, in their study about VT in social workers, Michalopoulos and Aparicio noted that higher levels of social support might serve as a protective factor against vicarious traumatization of social workers without a history of trauma but not of those who already experienced trauma (Michalopoulos and Aparicio 2012). Finally, a meta-synthesis study by Cohen and Collens found that increased stress levels in professionals working with trauma (in the context of secondary trauma) can be successfully managed through diverse organizational and personal interventions aimed to enhance individual coping strategies (Cohen and Collens 2013).

2.17 Mental Health Stigma and Stress

"*Stigma*" may be defined as a process involving labeling, stereotyping, separation, prejudice, and discrimination in a context in which social, economic, or political power is exercised to the detriment of members of a social group (Link and Phelan 2001). Noteworthy, stigma and discrimination are prevalent among individuals with

neuropsychiatric conditions (Bipeta et al. 2020; Kaushik et al. 2016; Kinson et al. 2018).

A mental health stigma is linked to a wide range of adverse consequences in stigmatized people, affecting their social, psychological, and physical health capacities. They include but are not limited to unemployment, housing problems, poor social adjustment, decreased self-esteem, and lower self-efficacy, poor interpersonal relationships with family and friends, and poor physical health (increased risk of obesity, unhealthy eating habits, and risky sexual behaviors) (Sickel et al. 2014; Rüsch et al. 2005; Forchuk et al. 2006; Perlick et al. 2001). A systematic review by Clement et al. also demonstrated that mental health stigma has a small- to a moderate-sized negative effect on help-seeking (Clement et al. 2015). Moreover, a study by Corrigan et al. on the impact of mental health stigma on healthcare providers reported that primary health care practitioners who endorsed stigmatizing characteristics of the patient were more likely to believe that one would not adhere to the prescribed treatment and hence, were less likely to refer one to a specialist or provide a refill of one's prescription (Corrigan et al. 2014).

Numerous studies demonstrated the interaction between mental health stigma and personal distress. For instance, Masuda et al. showed a significant positive correlation between mental health stigma and psychological distress (Masuda et al. 2009). In contrast, a study with young adult college students who endorsed having a past or current mental health diagnosis showed only a modest relation between levels of distress associated with prodromal psychotic symptoms and self-stigmatization. Interestingly, there was a more significant relationship between the intensity of distress derived from prodromal symptoms and self-stigma in students with low social support (measured by Friendships subscale of the Lubben Social Network Scale-Revised) than those with average and high social support (Denenny et al. 2015). The authors concluded that support from classmates and friends might act as a buffer of self-stigmatization-induced distress.

Mental illness stigma is also a potent source of distress in families of patients with mental disorders. A study with adult relatives of individuals with mental illness revealed that stigma was uniquely associated with caregiver distress, problems with empowerment, and difficulties in family functioning (Muralidharan et al. 2016).

Mental health stigmatization can also be detrimental for those individuals who stigmatize other people due to their mental health problems. For instance, Masuda et al. found that the course/origin component of stigma (marked by pessimistic views toward the cause, treatment prognosis, and recovery from a mental disorder) was associated with the psychological distress of the stigmatizer (Masuda and Latzman 2011).

2.18 Stress Vulnerability and Its Role in the Pathophysiology of Neuropsychiatric Disorders

The anatomical and functional connectivity of the brain is an essential determinant of the degree of stress resilience or vulnerability in an individual (Franklin et al. 2012). Brain circuitry can be remodeled by stressful experiences, causing functionally relevant morphological changes of the dendritic arbor, spine, and synapse within the AMG, HIPP, and PFC, and resulting in cognitive, emotional, and behavioral deficits (McEwen and Morrison 2013). Moreover, environmental challenges can precipitate psychiatric and neurological disorders in susceptible individuals (Zannas and West 2014).

Determining why certain people succumb to stress while others become stress-resilient is fundamental for understanding the neurobiology of stress susceptibility and resilience and is crucial for the successful development of effective treatments for stress-related disorders (Franklin et al. 2012). For instance, a study by Gilbertson et al. (2002) showed that a smaller HIPP volume might be a pre-existing condition that increases vulnerability to PTSD upon exposure to a traumatic event (Gilbertson et al. 2002). Similarly, other studies demonstrated that differences in HIPP volume might render individuals more or less vulnerable to adverse effects of stress on cognition and overall mental well-being (Lupien et al. 2018; Frodl et al. 2002; Narr et al. 2004).

Another significant factor related to increased stress vulnerability is early life adversity (such as poor parental care and physical abuse) (Lupien et al. 2018). Although the precise molecular mechanisms underlying these processes have not yet been fully elucidated, the initial evidence points to the HPA axis and its impact on brain morphology, functioning, and connectivity between regions involved in regulating the stress response (Lupien et al. 2018).

Genetic variations within the HPA axis (e.g., corticosteroid receptor polymorphisms), monoaminergic (e.g., dopamine D2 long receptor deficiency), and neurotrophic systems (e.g., variants of BDNF such as Val66Met polymorphism) could also be implicated and contribute to the increased stress vulnerability leading to the development of stress-related disorders (Shioda et al. 2019; Yu et al. 2012; DeRijk and de Kloet 2008; Hosang et al. 2014).

Epigenetic changes induced by gene–environment interactions exhibit a significant impact on stress responsiveness (Franklin et al. 2012). As reviewed by Zannas and West (Zannas and West 2014), specific environmental stressors can cause long-lasting epigenetic modifications (especially in genes relevant to the regulation of the HPA axis), carrying the potential to shape individuals' magnitude of stress responses. The same researchers also emphasized that these epigenetic variations might then be transferred and inherited by future generations, ultimately affecting their stress responses (Portela and Esteller 2010).

A study by Allen and Dwivedi published in 2020 showed that microRNAs could play a role in the maladaptive processes associated with early life adversities, ultimately leading to increased susceptibility of adolescents and adults to chronic

stress and stress-related disorders (Allen and Dwivedi 2020). Although the exact mechanism is still not completely clear, they suggested that observed microRNA changes might negatively affect various signaling systems regulating the stress response (Allen and Dwivedi 2020).

Menard et al. also emphasized the role of the innate and adaptive immune system in stress susceptibility, resilience, and coping. The researchers pointed out that pro-inflammatory cytokine signaling, peripheral monocyte infiltration, and microglial activation are significantly involved in the modulation of stress vulnerability (Ménard et al. 2017).

Although recent investigations made significant advancements in identifying biological substrates underlying the stress response, there are still numerous undetermined genetic and environmental factors that might be held accountable for inter-individual differences in reaction to stressful stimuli (Franklin et al. 2012).

2.19 Allostatic Load Index and Psychometric Stress Assessment

"*Allostatic load*" (AL) is defined as the "*wear and tear*" on the brain and body when primary mediators of "*allostasis*" (e.g., cortisol, adrenalin, cytokines) exert their noxious toll due to exposure to chronic stress conditions (Lupien et al. 2018; Picard et al. 2014).

According to McEwen, who coined the term, there are three types of physiological responses contributing to increased AL: (1) frequent stress/frequent activation of allostatic systems; (2) a failure to shut off allostatic activity after stress; and (3) inadequate response of allostatic systems leading to elevated activity of other, counter-regulated allostatic systems following stress exposure (McEwen 1998).

The "*Allostatic Load Index*" (ALI) quantifies the AL. The original index was based on ten physiological or physical measurements: 12-h urinary cortisol, adrenalin, and noradrenalin output; serum dehydroepiandrosterone sulfate (DHEAS), high-density lipoprotein (HDL) and HDL to total cholesterol ratio; plasma glycosylated hemoglobin; aggregate systolic and diastolic blood pressures; and waist-to-hip-ratio (Seeman et al. 1997). The original index score ranged from 0 to 10, with higher values indicating higher physiological strain and lower values indicating better adaptation to stress (Seeman et al. 2001). Moreover, the distributions for each biomarker were determined from observed data and divided into quartiles. Accordingly, observations in the high-risk quartile are scored as 1, while those in all other quartiles are scored as 0 (Edes et al. 2018). However, alternative criteria for calculating AL were also examined. One such alternative, using a stricter criterion, was based on a sum of the number of parameters for which the subject fell into the top (or bottom) 10% of the distribution (i.e., the group at highest "*risk*"). Another way of measuring it is to use an average of z-scores (how many standard deviations below or above the mean is a raw score) for each parameter. This approach showed the most robust effects (McEwen 2000).

A higher ALI score is typically associated with numerous antecedents (e.g., socioeconomic disadvantage, workplace stress, maladaptive personality traits, lifestyle behaviors, genetic polymorphisms) and health-related consequences (e.g., cardiovascular disease, obesity, hypertension, diabetes, cognitive decline, psychiatric symptoms, physical/mobility limitations, mortality, and other). Moreover, it represents a valuable tool that might help public and organizational health professionals to deepen their insights into the pernicious effects of chronic stress (Lupien et al. 2018; Edes et al. 2018; Juster et al. 2010).

Several psychometric instruments have been validated for assessing symptoms of stress, including the *Perceived Stress Scale* (PSS), which is one of the most popular tools for measuring the severity of psychological stress (Lee 2012).

Acknowledgments Translational Psychiatry Laboratory (Brazil) is supported by grants from CNPq (GZR), FAPESC (GZR), CAPES (GZR), Instituto Cérebro e Mente (GZR), and UNESC (GZR). GZR is a 2 CNPq Research Fellow. LMM is a CNPq Research Fellow.

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Chapter 3
Role of Glutamatergic Neurotransmission in the Pathophysiology of Stress-Related Disorders and Chronic Stress Response

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Abstract We begin this chapter with findings pointing out the interaction between exposure to stressful events and the occurrence of major psychiatric and neurological diseases, including Depression, Schizophrenia, and Neurodegenerative Disorders. The following sections present the impact of chronic stress on the glutamatergic system, ionotropic and metabotropic glutamate receptors, and excitatory amino acid transporters. The chapter continues with a description of animal models of chronic stress such as Chronic Mild and Restraint Stress, Chronic Social Defeat Stress, Chronic Subordinate Colony Housing, and Rat Cumulative Allostatic Load Measure developed to mimic the pathophysiology of human stress-related disorders. Their application in the translational development of glutamatergic treatments for psychiatric and neurological chronic stress-induced diseases is also discussed. The following section is dedicated to the impact of chronic stress on gene expression and epigenetic modifications in glutamatergic neuronal networks, followed by the part on stress-induced glutamate effects leading to oxidative and nitrosative neuronal stress and excitotoxicity. We conclude the chapter with the latest preclinical and clinical research insights on glutamate interactions with other neurotransmitters relevant to human stress response, such as serotonin, corticosteroids, GABA, and BDNF.

Keywords Glutamate · Chronic stress neurobiology · Glutamate receptors · Ketamine · NMDA receptors · AMPA receptors · Glutamatergic metabotropic receptors · Animal models of depression

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_3

3.1 Role of Glutamatergic Neurotransmission in the Pathophysiology of Stress-Related Disorders

3.1.1 Major Depressive Disorder

Major depressive disorder (MDD) is the most common mood disorder and a leading global cause of psychosocial disability (James et al. 2018). It is associated with depressive mood, general disinterest, psychomotor and vegetative symptoms, and cognitive impairment (American Psychiatric Association 2013). Genetic factors, combined with environmental influences such as stressful events, play significant roles in its onset (Cattaneo and Riva 2016; Krishnan and Nestler 2008).

Excitatory amino acids such as glutamate mediate both adaptive and harmful effects of stressors on the brain (Nasca et al. 2015a). Moreover, acute stress increases glucocorticoids, induces glutamate release, and affects glutamate receptors, glutamate clearance, and metabolism (Popoli et al. 2012). Multiple studies suggested that long-term augmentation of extracellular glutamate can lead to neuronal injury or death through the mechanism of glutamate-induced excitotoxicity (Olney and De Gubareff 1978; Olney et al. 1980).

Stress is affecting glutamate neurotransmission in region-specific ways. Numerous studies have reported enhanced chronic stress-related glutamate release and glutamate receptor expression, associated with reduced glutamate uptake/metabolism in the hippocampus (HIPP) (Sun et al. 2015; De Vasconcellos-Bittencourt et al. 2011). For instance, a study that directly compared stress-resilient to stress-susceptible mice displaying depressive-like behaviors indicated that enhanced HIPP glutamate expression was unique to depression-susceptible mice (Sun et al. 2015). Other studies showed a reduced chronic stress-related glutamate receptor expression in the medial prefrontal cortex (mPFC), which according to the authors, might represent a potential protective mechanism against excessive glutamate signaling and excitotoxicity in this brain region (Jett et al. 2017; Yuen et al. 2012).

Changes in glutamate receptors have also been observed in chronic stress-induced animal depression models. Several studies using animal models of depression reported that the chronic stress effects on dendritic remodeling were blocked by N-methyl-D-aspartate (NMDA) receptor antagonists (Popoli et al. 2012). Concerning changes in metabotropic glutamate receptors (mGluR) due to chronic stress conditions, Nasca et al. (2015b) identified mGluR2 as one of the biomarkers of stress susceptibility.

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) ionotropic receptors are also involved in stress-induced depression pathophysiology (Faye et al. 2018). For instance, Freudenberg et al. (2015) reported changes in AMPAR gene expression, especially those regulating synthesis of the GluR1 subunit proteins. Studies have shown that the non-specific deletion of the GluR1-containing AMPARs in forebrain neurons reduced immobility in the forced swimming and tail suspension tests while increasing the time animals spent in the open arms in the elevated plus-maze test (model used to assess anxiety-related behavior in rodents).

Increased animal entries in the center during the open field test model and the light compartment during the light-dark box test (another paradigm for assessing anxiety-related behavior in rodents) were also observed. Altogether, these findings strongly indicate AMPARs role in reducing depressive and anxiety symptoms (Fitzgerald et al. 2010; Maksimovic et al. 2014).

3.1.2 Anxiety Disorders

Anxiety disorders are among the most prevalent and disabling mental health illnesses (Erickson et al. 2009). They usually begin during childhood, resulting in significant suffering and disability, mainly due to their chronic and recurrent lifetime course (Kalin 2020). Anxiety disorders typically manifest as excessive fear and an increase in avoidance behaviors, often in response to a specific object or situation and usually in the absence of an actual danger (Shin and Liberzon 2010). Several interrelated limbic structures represent the anatomic core of fear and anxiety, consisting of specific nuclei of the amygdaloid complex, the septo-HIPP system, the periaqueductal gray matter, and certain areas within the hypothalamus (Deutch and Charney 1996). These brain structures serve to evaluate the extent to which environmental situations are threatening to the individual. At the same time, they contribute to establishing the appropriate patterns of behaviors associated with personal defense mechanisms (Millan 2003).

Several lines of evidence suggest that glutamatergic neurotransmission within the limbic system plays a pivotal role in the pathogenesis of anxiety disorders (Garakani et al. 2006; Vaquero-Lorenzo et al. 2009). Studies have consistently indicated that acute glutamate receptor-mediated activation, by either GABA disinhibition or CRF excitation, induces long-term synaptic plasticity and increases the excitability of basolateral amygdala (BLA) neurons (Sajdyk and Gehlert 2000; Shekhar et al. 2003). Lowery-Gionta et al. (2018) demonstrated that chronic stress dysregulated the BLA-PFC circuit by altering presynaptic glutamate release from BLA projections, suggesting this interaction as a potential mechanism of stress-induced anxiety. An interplay between the brain-derived neurotrophic factor (BDNF) and glutamate release in the pathophysiology of stress-induced anxiety was suggested by Chiba et al. (2012). They showed that BDNF-induced glutamate release was attenuated by exposure to chronic restraint stress, an animal model typically associated with stress-induced anxiety.

3.1.3 Substance Use Disorders

Substance use disorders (SUD) are predominantly chronic disorders characterized by a compulsion to use lawful or illegal substances, loss of control over their consumption, and continuous abuse despite adverse somatic and psychological consequences

(Cadet and Bisagno 2013). Chronic substance use is associated with neuroadaptations in brain reward pathways, which produce secondary psychiatric symptoms during acute and protracted drug-withdrawal states (McEwen 2000). These alterations primarily involve glutamatergic neuronal networks and manifest as increased emotional distress (Koob and Le Moal 1997; Sinha 2001), intensive craving symptoms, cognitive deficits, and maladaptive behaviors such as increased drug-seeking and extended substance use (Abé et al. 2013; Ernst and Chang 2008; O'Neill et al. 2015).

Animal models of stress and substance abuse reveal that previous exposure to stress increases the susceptibility of animals to the behavioral effects of psychostimulants and opioids and promotes drug self-administration (Esparza et al. 2012; Garcia-Keller et al. 2013). Studies have consistently suggested that regions with glutamatergic projections to the nucleus accumbens (NAc), such as the PFC, amygdala (AMG), and HIPP, may contribute to the effects of stress on behaviors related to substance use (Belujon and Grace 2011; Bagot et al. 2015). Kalivas (2007) reported that drug-seeking behaviors might be associated with a significant release of presynaptic glutamate from the PFC projections to the NAc, resulting in upregulation of postsynaptic AMPARs and downregulation of presynaptic mGluR2/3. Moreover, brain imaging studies using magnetic resonance spectroscopy to compare addicted individuals with controls have reported lower brain glutamate or Glx (glutamate+glutamine) levels in the mPFC and posterior cingulate cortex (PCC) (Zhang and Volkow 2019).

Studies in animal models of stress-related drug-seeking/use involve a complex interplay between several neurotransmitter systems. For instance, the effects of footshock stress on cocaine responding depended on glutamate release from dorsal PFC to NAc core projections (McFarland et al. 2004) and corticotropin-release factor signaling from the ventral tegmental area (VTA) (Wang et al. 2005; Williams et al. 2014), ultimately leading to an increase in the dopamine neurotransmission within NAc (Wise 2009). Along the same lines, Campioni et al. (2009) demonstrated that cold-water forced-swim stress altered the AMPAR/NMDAR ratio in the NAc shell, an effect that was reversed with the glucocorticoid receptor antagonist RU486. Moreover, noradrenergic signaling via alpha2 and beta2 receptors located in the dorsal bed nucleus of the stria terminalis was found to modulate glutamate transmission via alpha2-adrenergic receptors (Egli et al. 2005).

Taking into account all aforementioned findings, we agree with the suggestion provided by Greenwald that a direct pharmacological approach to the treatment of stress-related substance use by solely modulating the glutamatergic system might be challenging, as the significant interaction between glutamate and other neurotransmitters must be taken into account when designing future effective treatments for SUD (Greenwald 2018) (see Fig. 3.1).

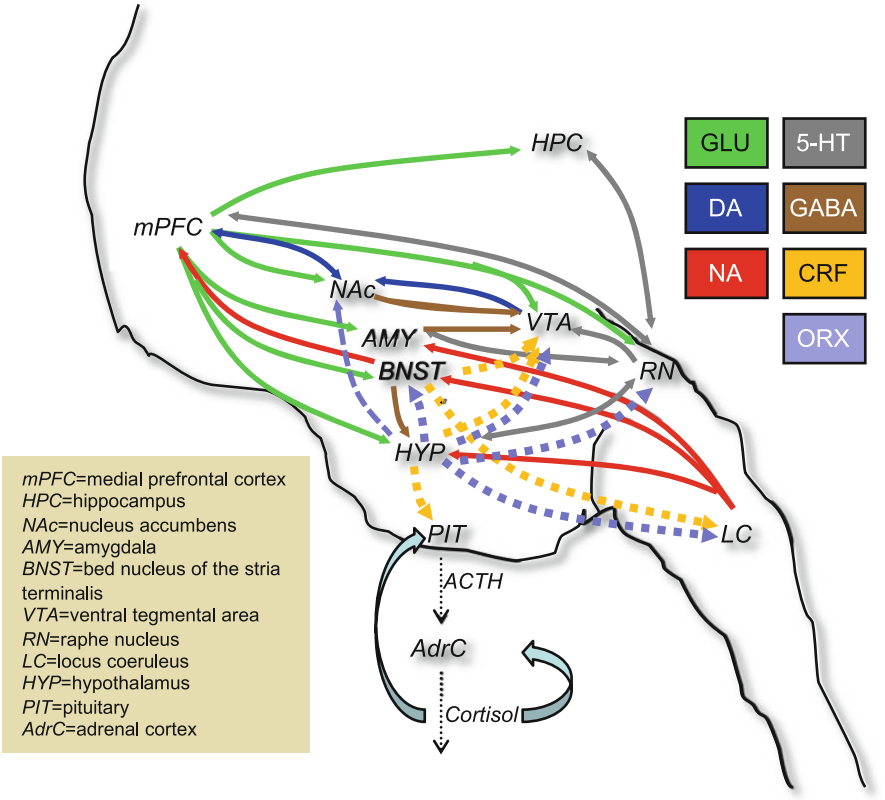

Fig. 3.1 Major neurochemical pathways integrating and underlying stress-potentiated drug-seeking/use behaviors. Glutamatergic neuronal pathways from the mPFC to other brain regions implicated in drug reward and addiction are highlighted in green [from Greenwald (2018)]

3.1.4 Somatization Disorders

Somatization disorders (SD) are characterized by chronic, medically unexplained, treatment-resistant symptoms, usually induced by severe or prolonged psychological distress. They are often associated with chronic physical pain, fatigue, and a sense of discomfort (Lipowski 1988). Symptoms are typically multiple and vague and may refer to single or several body systems, such as the cardiopulmonary, gastrointestinal, genitourinary, and musculoskeletal (Servan-Schreiber et al. 2000). Like other psychiatric conditions, somatization disorders result from the interplay between genetic factors, life events, and demographic variables such as ethnicity, education, and sex (Kirmayer and Young 1998).

It is widely acknowledged that stressful life events might precipitate a specific SD (Mai 2004). Craig et al. (1994) showed that during 38 weeks before the onset of symptoms, somatizers were more likely to have experienced at least one stressful

event. Moreover, studies have consistently reported that SD may be triggered by a traumatic childhood experience such as maternal deprivation, suggesting that both adverse relationships with significant others during childhood and personality traits might predispose an individual to develop SD during adolescence or adulthood (Henker et al. 2019; Lehmkuhl 2013).

There are currently only a few studies reporting on the involvement of the glutamatergic system in the pathophysiology of SD. One of these is a study by Fayed et al. (2012), which demonstrated a significant increase in the levels of Glx, a combined measure of glutamate (Glu) and glutamine (Gln), in fibromyalgia and, to a lesser extent, in SD, compared with controls. Other studies have shown that the changes in Gln levels in the posterior insula (Harris et al. 2009), left HIPP (Valdés et al. 2010), and anterior cingulate cortex (ACC) (Mullins et al. 2005) were highly correlated with the perceived intensity of pain (Fayed et al. 2012). Moreover, glutamate has also been implicated in chronic pain sensitization and potentiation (Dickenson et al. 2002).

Finally, Peterlik et al. (2017a) demonstrated that exposure to a chronic psychosocial stressor led to inhibition of mGluR5, suggesting that this receptor could be involved in the pathophysiology of SD and might be considered a potential target when developing novel glutamate-based treatments for these increasingly prevalent disorders.

3.1.5 Post-traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) is a chronic, debilitating psychiatric disorder that can develop after exposure to highly stressful and traumatic experiences (Friedman et al. 2007; Hollifield et al. 2002). Several neuroimaging studies showed functional and structural anatomical changes in patients with PTSD, mainly in the prefrontal subregions such as ACC, orbitofrontal, and subcallosal cortex (Lanius et al. 2002), and their afferent and efferent glutamatergic projections (Moghaddam 2002). Alterations in glutamate neurotransmission have also been implicated in morphological gray matter changes, mainly caused by significant elevations in extrasynaptic glutamate due to excessive glial loss, leading eventually to glutamate-induced excitotoxicity (Kassem et al. 2013).

Studies have shown that HIPP atrophy plays a significant role in the neurobiology of PTSD (McEwen 2007; Woon et al. 2010) and might be partly caused by stress-induced glutamate excitotoxicity, leading to neuronal injury and loss, manifested as re-experiencing of traumatic events, and deficits in memory and attention (Rosso et al. 2017). Rosso et al. (2017) reported that both glutamate and the neuron marker N-acetyl aspartate (NAA) were significantly increased in the right HIPP of PTSD patients compared to controls. They also noted that these changes proportionally correlated with the severity of re-experiencing symptoms (Rosso et al. 2017).

Concerning mGluR role in PTSD, several studies demonstrated that mGluR5 activity underlies stress-induced fear conditioning (Tronson et al. 2010) and that

antagonism of mGluR5 blocks the acquisition and expression of a conditioned fear response (Schulz et al. 2001). Multiple findings from acute stress and PTSD studies strongly emphasize the importance of mGluR5 and glucocorticoid system interactions in the neurobiology of both acute stress disorder (ASD) and PTSD. Interestingly, while the results from the acute stress studies showed a decrease in the number of mGluR5 and upregulation of the glucocorticoid system, upregulation of mGluR5 and reduction in glucocorticoid signaling was noted in individuals with PTSD (Holmes et al. 2017).

Finally, several studies observed a significant association between glutamate decarboxylase 1 (GAD1) gene polymorphisms (the gene encoding enzyme for catalyzing the production of gamma-aminobutyric acid from L-glutamic acid) and increased severity of PTSD symptoms in combat Veterans, implying that this association may represent a shared genetic risk factor (Haxhibeqiri et al. 2019; Bountress et al. 2017).

3.1.6 Schizophrenia

Schizophrenia is a complex multifactorial disorder that manifests through positive, negative, and cognitive symptoms. It is hypothesized that it develops due to the interaction between genetics and environmental stressors (Mizrahi et al. 2014). Initial insights on the implication of glutamate and NMDAR in the neurobiology of schizophrenia stem from the similarity between symptoms observed in individuals during the acute schizophrenic episode and psychosis induced by Phencyclidine (PCP), a non-competitive NMDAR antagonist (Toriumi et al. 2016).

Dickenson et al. (2002) reported that stress-induced changes in the glutamatergic neurotransmission might be involved in the pathogenesis of schizophrenia. Multiple studies have shown that the region most commonly affected is the HIPP, which exhibits reduced NMDAR expression in individuals with schizophrenia (Beneyto et al. 2007; Vrajová et al. 2010). Moreover, Réus et al. (2017) reported on the adverse effects of Ketamine, an antagonist of NMDAR, which increased the levels of oxidative stress in an animal model of schizophrenia.

Studies have also shown a relationship between AMPARs activity and symptoms of schizophrenia. AMPARs are responsible for fast glutamate transmission, neuronal circuit remodeling, and higher-order cognitive functions such as learning and memory. Lin and Lane demonstrated that abnormalities in AMPAR trafficking might contribute to cognitive impairments observed in patients with schizophrenia (Lin and Lane 2019).

A study by Fell et al. (2008) was one of the first to report on the role of mGluR2 in the downregulation of excessive dopamine release in an animal model of schizophrenia. Similarly, Patil et al. demonstrated that an mGluR2/3 agonist, which downregulates disinhibited glutamate release, exhibited antipsychotic properties (Patil et al. 2007).

Several neuroimaging studies also demonstrated that the glutamate levels in the ACC were higher in patients with schizophrenia when compared with healthy individuals (Demjaha et al. 2014; Mouchlianitis et al. 2016). However, other studies found no difference in glutamate concentrations between chronic schizophrenia patients and control subjects (Bustillo et al. 2011; Kraguljac et al. 2012). Studies have also linked alterations in glutamate/glutamine levels to the development of schizophrenia (Egerton et al. 2012; Kumar et al. 2020). Along the same lines, Kraguljac et al. (2012) showed higher medial-frontal glutamate/glutamine levels in high-risk subjects, while Egerton et al. (2012) found higher levels of glutamate and Glx (a combined measure of glutamate and glutamine) in the ACC of symptomatic patients with schizophrenia compared to those in remission.

3.1.7 Neurodegenerative Disorders

Neurodegenerative disorders are illnesses caused by progressive loss of neurons, which contrasts with static neuronal death in severe metabolic disorders and intoxication (Grimm and Eckert 2017).

Multiple studies have reported the negative impact of stress on the aging process and progressions of neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Pardon and Rattray 2008; Peña-Bautista et al. 2020). Concerning AD, Tsolaki et al. (2010) noted that most patients with AD typically present with a history of multiple stressful events, such as problems due to lack of money, or the death of a spouse or a partner, before the onset of dementia. The same applies to PD, as stressful life events, such as divorce, death of a child, or long-term unemployment, may precipitate its development (Hemmerle et al. 2012). Gibberd and Simmonds (1980) demonstrated that prisoners of war had a much higher incident rate of PD development, 35 years after release from prison, than controls. Along the same lines, emotional stress can transiently increase motor symptoms in patients with PD (Macht et al. 2007).

Studies have shown that early-life stress can also increase the vulnerability to neurodegenerative disorders, leading to alterations in neuronal morphology and dysregulation of neurotransmitters systems (Desplats et al. 2020). For instance, Barros et al. (2006) reported that specifically adult males born to stressed mothers showed a reduction in dendritic arborization, while Berger et al. (2002) demonstrated that the same changes were accompanied by an increase in ionotropic and metabotropic glutamate receptors in specific brain structures, such as the frontal cortex (FC), HIPP, and striatum. An increased concentration of vesicular glutamate transporters type 1 (vGluT1) has also been observed in the FC and HIPP of prenatally stressed rats (Adrover et al. 2015).

Considering PD, Castro and Zigmond (2001) reported on the effects of glutamate on substantia nigra pars compacta of laboratory animals, resulting in the increased striatal release of dopamine during stress exposure and enhanced vulnerability of neurons to future neurotoxic events within the nigrostriatal pathway. In humans,

Mironova et al. (2018) showed that the concentration of serum glutamate in PD patients was higher than in healthy subjects, while Mellone et al. (2015) noted that the number of GluN2A subunits and GluN2A/GluN2B subunit ratio of NMDARs were increased in PD patients in comparison to controls.

In conclusion, the results of these and other studies strongly support the role of chronic stress in the initiation and progression of major neurodegenerative disorders such as AD and PD.

3.1.8 Suicidality

Suicide is a significant public health concern and a leading cause of death in most societies, claiming the lives of over 800,000 individuals annually (World Health Organization 2017). It is the second leading cause of death among the youth population and the seventeenth leading cause of death overall (Bernstein et al. 2013). Moreover, it is a complex and heterogeneous phenomenon associated with multiple factors, such as individual psychopathology, personality traits, early-life adversity, and chronic stressful life events (Lutz et al. 2017).

A growing body of evidence suggests an involvement of the glutamatergic neurotransmitter system in suicidal behavior (Bernstein et al. 2013), serving as a mediator between suicidal vulnerability and external stressors (Courtet et al. 2016). The stress-induced suicide hypothesis is based on the notion that a systemic low inflammatory state induced by chronic stress promotes HPA axis dysregulation and activates the kynurenine pathway that degrades the tryptophan, thus increasing neuroactive excitotoxic metabolite quinolinic acid (Courtet et al. 2016; Schwarcz et al. 2012). Subsequently, through activation of NMDAR containing the combination of NR1 + NR2A and the NR1 + NR2B subunits, quinolinic acid induces neurotoxicity of neurons located within the HIPP, striatum, and neocortex (Prado De Carvalho et al. 1996). Guillemin (2012) has shown that quinolinic acid increased glutamate release from neurons, inhibited its uptake by astrocytes, and reduced the activity of astroglial glutamine synthetase.

Concerning mGluRs, Sequeira et al. (2009) reported a decrease in mGluR3 receptors in the PFC of people who died by suicide than healthy subjects. An increase in mGluR1, 2, and 3 receptor gene expression was noted in the ACC of suicidal patients with depression, while ionotropic glutamate receptor kainate type subunit 1 (GRIK1) gene expression was significantly enhanced in suicide completers compared to controls, especially if present in the region of the ACC (Zhao et al. 2018). As for AMPARs, upregulation in the caudate (Thomas Noga et al. 1997) and lower AMPA GRIA3 gene expression in the PFC (Sequeira et al. 2009) were the main findings of suicidal behavior.

In contrast, several studies found no differences between suicide victims and controls regarding glutamate levels in forebrain areas and neocortical tissues (Korpi et al. 1988; Palmer et al. 1994). In contrast, alterations in the glutamate-glutamine cycle were observed by Sequeira et al. (Sequeira et al. 2009). The authors noted a

significant downregulation of the glutamine synthetase (an enzyme involved in several metabolic pathways in the brain, including the glutamine-glutamate-GABA cycle and brain ammonia detoxification) in areas of PFC and HIPP of depressed patients with suicidal tendencies. Glutamine synthetase (GS) was also significantly less expressed in patients with schizophrenia, who died from suicide compared to those who died from other causes (Kim et al. 2007). Finally, Kim et al. (2007) demonstrated that GS expressed in glial cells was increased in the mediodorsal thalamus, dorsolateral prefrontal, and orbitofrontal cortex of suicide victims with schizophrenia, suggesting that cerebral GS deficit might serve as an indicator of a future suicidal behavior (Jimenez-Trevino et al. 2020).

3.1.9 Chronic Physical Health Conditions

The prevalence of chronic diseases is increasing exponentially, advancing globally and pervading all socioeconomic classes (Petersen 2000). According to the latest report by the World Health Organization (WHO) (World Health Organization 2017), the most prevalent chronic diseases like cardiovascular diseases, cancer, chronic obstructive pulmonary disease, and type two diabetes mellitus (T2DM) are sharing similar and preventable biological risk factors, notably high blood pressure, increased levels of blood cholesterol and glucose, and obesity. An unhealthy diet, poor nutrition, physical inactivity, smoking, excessive alcohol use, and psychosocial stress are considered the most significant behavioral risk factors (Hathaway 2018). Recently, several epidemiological studies have demonstrated that constant life pressure typical for modern societies is one of the most important contributors to the onset of a specific chronic disease, such as T2DM and various cardiovascular diseases (Krantz et al. 2000; Paradies 2006).

It is common knowledge that stressful events may affect the onset and overall metabolic control in patients with DM (Mishra et al. 2020). Moreover, stress-related factors, such as stressful events at work, traumatic life experiences, depression, and other mental health problems, can independently be responsible for developing DM (Kelly and Ismail 2015).

Glutamatergic neurotransmission plays a critical role in human cortical synaptic plasticity, learning, and memory (Huang et al. 2007) and is often affected in type one (T1DM) and T2DM (Trudeau et al. 2004). Experiments in non-obese diabetic mice suggested that upregulation of NMDAR is associated with the early stages of DM (Trudeau et al. 2004). Andersen et al. (2017) demonstrated a specific HIPP impairment in glutamate and glutamine metabolism in one of the most widely used T2DM mouse models (db/db mouse). Liu et al. (2019a) showed that higher levels of glutamate and lower levels of glutamine in plasma were associated with an increased risk of T2DM. Along the same lines, Fried et al. (2019) observed that disruption of glutamine metabolism occurs already in prediabetic individuals, manifested by lower levels of glutamine and a lower glutamine/glutamate ratio in cortical regions. Finally, a study by Wiegers et al. (2019) reported that glutamate levels were higher

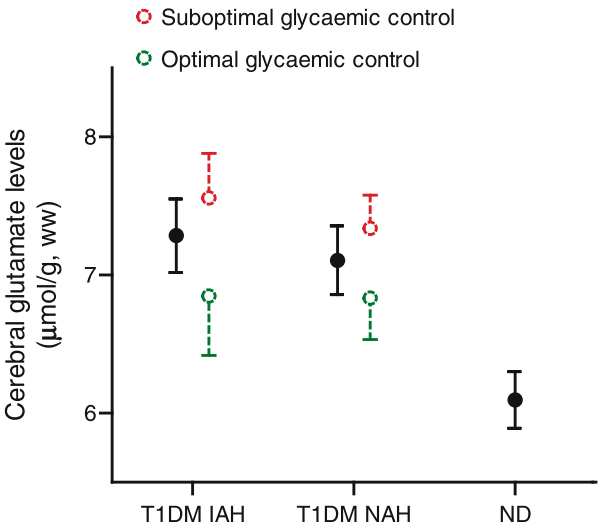

Fig. 3.2 Cerebral glutamate levels in participants with T1DM and non-diabetic (ND) controls. Cerebral glutamate levels were significantly higher ($p < 0.01$ T1DM vs. ND controls) in individuals with T1DM than ND controls. Group means are depicted in black circles; impaired awareness of hypoglycemia (IAH); normal awareness of hypoglycemia (NAH) [from Wiegers et al. (2019)]

in the brains of participants with T1DM compared to non-diabetic controls and suggested that glutamate might potentially serve as an early biomarker of hyperglycemia-induced cerebral complications of T1DM (Wiegers et al. 2019) (see Fig. 3.2).

Altogether, these findings strongly indicate that glucose intolerance in the early stage of disease might be related to a disruption of glutamine metabolism and glutamatergic neurotransmission.

In conclusion, while results from past and recent epidemiological studies did establish a more or less direct relationship between chronic stress and occurrence of major somatic illnesses, future investigations will need to shift focus toward identifying interactions between neuronal networks implicated in the neurobiology of chronic stress on one side, and affected body organs on the other side, thus paving the way for replacement of current symptomatic treatment interventions, with much more effective and sophisticated modalities that will prevent the onset of chronic physical health conditions.

3.2 Role of Glutamatergic Neurotransmission in Chronic Stress Response

3.2.1 Role of NMDAR, AMPAR, and mGluRs in the Pathophysiology of Chronic Stress

Chronic stress is associated with mental health conditions such as anxiety and depression, which are increasingly prevalent and dramatically impact global health and the economy (De Kloet et al. 2005). Several neurotransmitter systems are

implicated in the pathophysiology of the human stress response; one of them is glutamatergic. The neurotransmitter glutamate exerts its effects through cell surface receptors. In mammals, four families of glutamate receptors have been identified: (1) α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA); (2) kainate or kainic acid receptors (KARs); (3) N-methyl-D-aspartate (NMDA); and (4) metabotropic receptors (mGluR), which are coupled to the G- protein (classified 1–8).

Research studies have consistently shown the involvement of glutamatergic receptors, including ionotropic (iGluR), AMPAR and NMDAR, and mGluR, in the neurobiology of animal models of chronic stress. While most studies previously focused on the role of iGluR on the chronic stress response, recently, the scientific community's attention shifted toward mGluR. For instance, a study by Peterlik et al. reported downregulation of mGluR7 in the PFC after exposure to chronic psychosocial stressors induced by subordinate colony housing during 19 days. They also demonstrated that mGluR7 deficiency reduced behavioral symptoms of anxiety and immune system changes caused by chronic stress (Peterlik et al. 2017b). The same research group showed that the blockage of mGluR5 mitigated maladaptive chronic stress consequences (Peterlik et al. 2017a). Others also investigated mGluR role in the regulation of synaptic plasticity during chronic exposure to stressful situations. Indeed, Li et al. recently reported that behavioral alterations due to chronic stress were associated with an upregulation of the Homer1 (Homer1 is a postsynaptic scaffolding protein located in the postsynaptic density, which prominently links group one (I) mGluRs to its downstream targets)-mGluR5 signaling pathway (Li et al. 2019; De Bartolomeis and Tomasetti 2012). Interestingly, Sengupta and Chattarji (Sengupta et al. 2016) demonstrated that repeated chronic exposure to stress, rather than a single stressful experience, is required to induce HIPP changes mediated by mGluRs.

The interplay between external stress and illicit substance use is a well-established phenomenon. Indeed, a study demonstrated that GluA2 knock-in mice (mice with a mutation of K882 within the intracellular C terminus of the GluA2 subunit of the AMPAR) exhibited an increased stress response following cocaine self-administration (Ellis et al. 2017). Along the same lines, the disruption of GluA2 phosphorylation increased the vulnerability of mice to stress-induced reinstatement of cocaine-seeking and cocaine-conditioned reward behaviors (Ellis et al. 2017).

Multiple studies have shown that glutamate receptor modulators could positively affect behavioral, physiological, and molecular changes induced by chronic stress (Mishra et al. 2021; Réus et al. 2012). For example, Memantine, an antagonist of NMDAR approved for AD treatment, exerted an antidepressant-like effect by preventing HIPP mitochondrial dysfunction and stress-induced memory impairment in the rats subjected to chronic unpredictable stress (CUS) (Mishra et al. 2021). Along the same lines, Réus et al. (2012) demonstrated that Memantine reversed anhedonia and the increase of adrenal gland weight induced by chronic mild stress (CMS), normalized corticosterone levels, and increased BDNF levels in the PFC. Classical antidepressants can also restore a decrease in 2A and 2B subunits of the NMDAR within the FC induced by CMS (Martín-Hernández et al. 2019). Ketamine,

an NMDAR antagonist with antidepressant properties, has also proven effective in abolishing chronic stress responses in laboratory animals. Indeed, in rats submitted to CMS, subanesthetic Ketamine doses reversed behavioral changes regulated by the hypothalamic-pituitary-adrenal (HPA) axis and promoted antioxidant effects in chronically stressed rats (Maciel et al. 2018; Garcia et al. 2009). Pałucha-Poniewiera et al. (2021) reported that administration of an mGluR2/3 receptor antagonist, LY341495, induced similar antidepressant-like effects in the CUS model. Moreover, its combination with Ketamine showed synergistic effects, probably due to sharing of a similar mechanism of action, through the activation of mammalian target of rapamycin (mTOR) pathway (Pałucha-Poniewiera et al. 2021). Finally, in a study by Blien et al., administration of another amino acid, methionine, usually decreased in patients with depression, resulted in increased animal resilience to chronic stress and rescued social avoidance behaviors through an epigenetic mechanism involving histone methylation of cortical NMDAR (Bilen et al. 2020).

3.2.2 Role of Excitatory Amino Acid Transporters in Chronic Stress

Excitatory amino acid transporters (EAATs) encompass a class of five transporters EAAT1, EAAT2, EAAT3, EAAT4, and EAAT5 (Malik and Willnow 2019), expressed in neurons and glial cells. EAATs, like glutamate receptors, play an essential role in regulating synaptic plasticity and neurotransmission. Together with ionotropic and metabotropic receptors, they are considered molecular targets for developing novel glutamatergic treatments for central nervous system (CNS) disorders (Bunch et al. 2009). As transmembrane proteins, they continuously recycle glutamate released into the synapse back into presynaptic neurons, thus preventing neurotoxicity and the death of neurons and enabling their undisrupted activity and survival (Malik and Willnow 2019). EAAT1 and EAAT2 are the most extensively studied glutamate transporters. Their dysfunction is linked to the etiologies of numerous psychiatric disorders, including schizophrenia (Parkin et al. 2018; Spencer and Kalivas 2017; O'Donovan et al. 2017).

The role of EAATs in the neurobiology of chronic stress has been consistently reported. For instance, in an animal experiment, rats subjected to CMS had a lower expression of EAAT1 and EAAT4 in the FC, which was restored after the treatment with antidepressant drugs (Martín-Hernández et al. 2019). Along the same lines, increased EAAT2 mRNA and protein expression in the HIPP was found in rats subjected to chronic restraint stress; however, their subsequent treatment with an antidepressant Tianeptine completely reversed the changes (Reagan et al. 2004). Fontella et al. (2004) demonstrated that repeated restraint stress for 40 days augmented neuronal presynaptic glutamate uptake and basal and K+-stimulated glutamate release when measured 24 h after the last stress session. In rats with an anxious phenotype subjected to CMS, a decrease in EAAT1 expression in the HIPP was also

noted (Réus et al. 2015a). Administration of subanesthetic doses of Ketamine to rats exposed to CUS upregulated the expression of EAAT2 and EAAT3, reduced the concentration of extracellular glutamate in the HIPP, and decreased depressive-like behaviors (Zhu et al. 2017). Using the same animal model, Yu et al. (2019) showed that downregulated astroglial EAAT2 inhibited synaptic plasticity in the dentate gyrus of the HIPP simply by reducing glutamate metabolism. A study in mice exposed to CUS revealed lower protein levels of EAAT1 and EAAT2 in the PFC (Liu et al. 2019b). Interestingly, these changes were entirely restored following treatment with an SSRI antidepressant Fluoxetine and Xiaoyaosan (a Chinese herbal formula with potential antidepressant properties) (Fig. 3.3) (Liu et al. 2019b).

3.2.3 Glutamate Role in Animal Models of Chronic Stress-Induced Depression

3.2.3.1 Glutamate Role in Chronic Mild Stress Animal Model

Mental disorders (e.g., depression, anxiety) are often triggered by exposure to stressful events. Therefore, to gain insights into their etiology, several animal models relying on exposure to stress have been developed. These paradigms typically lead to changes in emotional behaviors reminiscent of a depressive or anxious phenotype, including anhedonia, despair, fear, lack of motivation, or social anxiety. Glutamatergic alterations have been observed in human studies of various stress-related disorders and behaviors, including depression, panic disorder, PTSD, and trait anxiety. Some of these findings have been reproduced in rodent models of stress-induced emotional disturbances (see Page and Coutellier (2019) for a review), and proteomic analyses combining animal models of depression and post-mortem tissue specimens obtained from depressed patients altogether support the appropriateness of animal models to study chronic stress-induced psychiatric disorders (Carboni et al. 2016). Even though certain controversies regarding the use of behavioral tests in rodents to assess, for instance, their level of "depressive" behavior (Reardon 2019), have recently emerged, most findings based on rodent models of stress still support their translational value. The effects of acute stress on glutamate neurotransmission have been well documented, specifically in brain regions regulating mood and emotions like the PFC and the HIPP. Experimental evidence from animal studies mainly supports a rapid increase in glutamate release in response to acute stress (see Popoli et al. (2012) for a review).

In comparison, the effects of chronic mild stress (CMS) on glutamatergic neurotransmission are less clear. Still, several research studies suggest an opposite impact than that observed after exposure to acute stress models (Musazzi et al. 2015). In addition, differences in chronic stress protocols contribute to variability in results; other factors such as age at exposure to stress and sex also explain differences in findings.

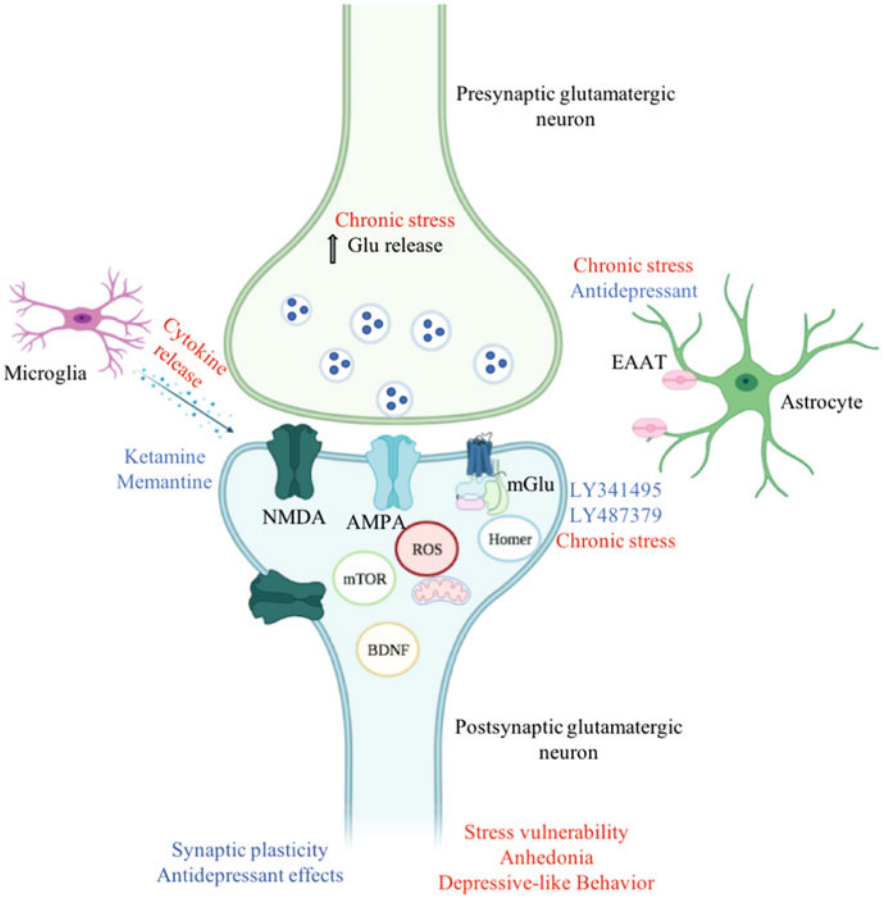

Fig. 3.3 Effects of chronic stress on the glutamatergic system. Chronic stress increases glutamate (Glu) neurotransmission, decreases the concentration of EAAT in the astrocytes, and leads to excessive microglial activation followed by the release of cytokines and other inflammatory mediators. These mechanisms mediated by iGluR and mGluRs further increase reactive oxygen (ROS) and nitrogen species (RNS), promote mitochondrial dysfunctions and decrease BDNF. In contrast, NMDA antagonists, Ketamine and Memantine, and mGluRs modulators (LY341495 and LY487379) reduce oxidative stress and increase BDNF levels, promoting neuronal synaptic plasticity changes leading to sustained antidepressant effects. Images were extracted from the Biorender app

CMS and Glutamate Neurobiology

The most common rats and mice CMS paradigm is the chronic unpredictable mild stress (CUMS) protocol. CUMS is widely used in adult rodent models to induce anxiety- and depressive-like phenotype and assess the physiological and neurochemical correlates of these behavioral changes. This paradigm consists of daily exposure to alternating mild stressors over several weeks. It consistently results in weight loss,

anhedonia, disrupted sleep patterns, reduced locomotor activity, and decreased motivation, all reminiscent of various aspects of depression (Willner 2017). Metabolomic analyses identified that the metabolites altered by CUMS are mainly involved in the glutamine-glutamate metabolic system (Ni et al. 2008; Xu et al. 2020). These findings were additionally verified by direct measures of glutamate/glutamine levels in the HIPP and PFC (using 1H magnetic resonance spectroscopy or high-performance liquid chromatography-mass spectrometry analysis). Glutamate levels are primarily increased by CUMS, while glutamine levels are decreased (Chen et al. 2019a; Ding et al. 2017). The increase in glutamate is likely due to abnormal glial functions (Banasr et al. 2010) and reduced reuptake of glutamate, evidenced by reduced EAAT 1 and 2 (Liu et al. 2019b; Ding et al. 2017). Altogether, the main findings point toward increased glutamatergic signaling induced by CUMS through an increase in glutamate release and a reduction in synaptic clearance (Hill et al. 2012) (see Fig. 3.4).

CUMS-induced changes at the level of glutamatergic postsynaptic receptors are less clear. For example, in the PFC, CUMS reduced levels of GluN2B subunits of NMDAR (Guo et al. 2016). Jiang et al. observed a decrease of GluN2A subunits in the HIPP (Jiang et al. 2019), while another group reported opposite results (Calabrese et al. 2012). The whole HIPP vs. ventral HIPP level of analysis might explain this discrepancy in findings. GluN1 and GluN2B subunits are consistently found to be increased in the HIPP after CUMS exposure (Jiang et al. 2019; Calabrese et al. 2012), while a lack of phosphorylation of the GluN2B subunit in the HIPP after CUMS has been related to the cognitive deficits observed after CUMS (Calabrese et al. 2017).

Changes in AMPARs have also been studied in the HIPP. The AMPAR GluR1 subunit is decreased by CUMS (Gao et al. 2017; Xiao et al. 2019), while AMPAR GluR2 and GluR3 are increased (Lin et al. 2018a).

CMS and Drug Treatment

Pharmacological interventions targeting glutamatergic transmission further validate the role of CMS-induced changes on glutamatergic receptors expression and their contribution to CMS-induced depressive- and anxiety-like behaviors. Activation of AMPARs using an AMPAR potentiator, or drugs that reverse the decrease in GluR1 expression induced by CUMS, reversed some aspects of depressive-like behaviors in mice (e.g., weight loss, immobility in the forced-swim test) (Xiao et al. 2019; Farley et al. 2010). Similarly, drugs that normalize NMDAR GluN2B subunit levels in the PFC (the selective SIRT2 inhibitor 33i, or YY21) have also demonstrated antidepressant effects (Guo et al. 2016; Erburu et al. 2017).

Studies using NMDAR antagonists strongly support a disrupted glutamatergic transmission in CMS-induced emotional deficits. For instance, Memantine administration reversed CMS-induced anhedonia, adrenal gland hypertrophy, and an increase in corticosterone levels (Réus et al. 2012). Along the same lines, Ketamine, the latest FDA-approved treatment for severe, treatment-resistant depression,

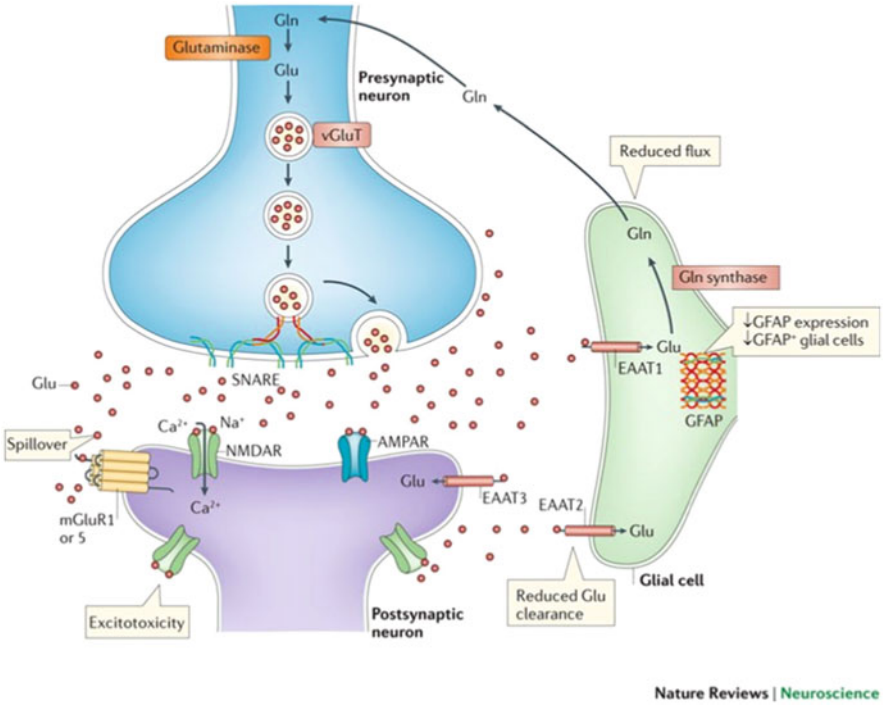

Fig. 3.4 Accumulating evidence suggests that chronic stress significantly affects glial cell function. Several studies have demonstrated decreases in the glial fibrillary acid protein (GFAP) expression and the number of GFAP-expressing glial cells in the HIPP and PFC following exposure to chronic stress. Chronic stress may also impair the ability of microglia to effectively clear synaptic glutamate (Glu) through glial EAATs. This impairment may lead to Glu spillover, ultimately increasing activation of extrasynaptic glutamate receptors and resulting in excitotoxicity. This paradigm has been proposed to occur in several neurodegenerative disorders and possibly after exposure to chronic stress. Finally, chronic stress may decrease flux rates through the glutamate-glutamine (Gln) cycle, resulting in reduced glutamate metabolism; vGluT, vesicular glutamate transporter [from Popoli et al. (2012)]

represents the main proof-of-concept of glutamate involvement in CMS-induced impairment in affective disorders. Several preclinical animal studies support the rapid antidepressant properties of this drug: mice and rats exposed to CUMS and treated with a single dose of Ketamine displayed attenuated depressive- and anxiety-like behaviors, lasting up to 8 days post-injection. These behavioral changes paralleled with the restoration of the CUMS-induced decrease in frequency and amplitude of excitatory synaptic currents (EPSCs) in the PFC, including the normalization of glutamate release in the HIPP (Li et al. 2011; Ma et al. 2013; Tornese et al. 2019) (see Fig. 3.5). Unfortunately, the psychedelic side effects and high-addiction potential associated with Ketamine limit its clinical use.

Other glutamatergic targets have also been validated for their antidepressant properties. In that sense, antagonism of the mGluR 2/3 produced rapid and

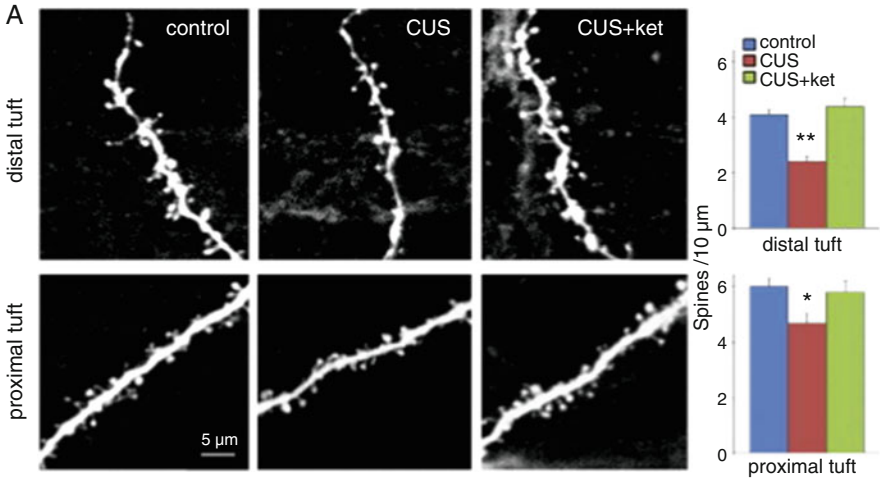

Fig. 3.5 Chronic unpredictable stress (CUS) exposure decreases spine density in PFC layer V pyramidal cells: rapid reversal by Ketamine. Animals were exposed to CUS for 21 days and then received Ketamine injections (10 mg/kg, intraperitoneal). Twenty-four hours later, slices of the PFC were prepared for whole-cell recordings, followed by neurobiotin labeling and post hoc two-photon microscopy image of the neurobiotin-labeled layer V pyramidal cells. Representative images are shown of high magnification Z-stack projections of distal and proximal segments of the layer five (V) pyramidal cell apical tuft dendrites (scale: 5 µm). The density of spines was analyzed using Neurolucida Explorer (version 9; MBF Bioscience, Williston, Vermont), and the results are the mean ± SEM (~12 cells from four rats in each group; $*p < 0.05$; $**p < 0.01$, analysis of variance). CUS decreased spine density of both distal and proximal segments of the apical tuft. This deficit was entirely reversed by Ketamine treatment (bar graphs to the right of images) [from Li et al. (2011)]

long-lasting (up to 10 days) anti-anhedonia effects in CUMS-exposed rats (Dwyer et al. 2013). At the same time, an mGluR5 negative allosteric modulation combined with a decreased expression of the mGluR7 rescued HPA axis abnormalities and anxiety-like behaviors induced by chronic subordinate colony housing, another animal paradigm used to assess the effects of chronic stress (Peterlik et al. 2017a, b).

Other pharmacological targets have also been tested to rescue depressive- and anxiety-like behaviors in CMS rodent models. Downstream effects of those compounds involve changes in the glutamatergic system, although not always, suggesting that variations in glutamatergic transmission do not solely drive emotional deficits. For instance, serotonin-selective reuptake inhibitors (SSRI) antidepressants, affecting primarily serotonergic transmission, like Fluoxetine or Escitalopram, rescued anhedonic, despair, and anxiety-like behaviors in CMS-exposed rodents (Mutlu et al. 2012; Binnetoglul 2019; Dong et al. 2015), and corrected the decrease in glutamate reuptake time in the HIPP (Binnetoglul 2019). Similarly, taurine, an amino acid with antidepressant-like effects, inhibited anhedonia and anxiety-like behaviors in rats exposed to CUMS by simultaneously

affecting HIPP glutamate, brain monoamines, corticosterone, and neurotrophic factor levels (Wu et al. 2017).

In contrast, Mirtazapine, a noradrenergic and serotonergic agonist, reversed CMS-induced anhedonia and GABA deficits in the NAc in rats, without reference to potential modulation of the glutamatergic system (Kamal 2013).

CUMS vs. Other Stress Models

CUMS paradigm is the central paradigm used in preclinical research to decipher the underlying causes and mechanisms of stress-induced emotional dysregulations. Its uniqueness is mainly ascribed to the "unpredictability" of the stressors used in the protocol, which is believed to play a significant role in its effects on the brain and behaviors. Predictable chronic stress studies further support this observation (e.g., 5-min daily restraint over a prolonged period), revealing that such exposures provide resilience to subsequent stressful exposures (Suo et al. 2013; Dang et al. 2019). However, researchers also reported similarities between the CMS and chronic restraint stress (CRS) effects on glutamatergic neurotransmissions, such as reduced glutamatergic transmission in the central AMG (Grillo et al. 2015). Changes in expression of the NMDAR and AMPAR subunits in the HIPP were observed in both models (Pacheco et al. 2017). Other researchers indicated that the specific nature of chronic stress and type of stressor could also drive behavioral and biological changes between the two models. For instance, metabolic bioinformatic analysis of the HIPP showed that physical stress (like learned helplessness or CUMS) is associated with changes in lipid and glutamate metabolism. In contrast, psychological stress (like chronic restraint or social defeat stress) mainly affects cell signaling, cellular proliferation, and neurodevelopmental changes (Liu et al. 2018).

Limitations of CMS Studies

From the studies reported here, it is evident that glutamatergic transmission is susceptible to chronic stress and drives some of the effects of chronic stress on emotional impairments. However, the complexity of these changes makes it challenging to find pharmacotherapeutic approaches that can consistently and reliably help in reducing symptoms of stress-related disorders like depression or anxiety. We described above that CUMS alters glutamatergic transmission differently in the PFC and HIPP, two brain regions directly involved in mood regulation. Other brain regions are also implicated in emotion regulation but have been, in comparison, poorly studied. For instance, diminished glutamatergic transmission in the ventral part of the periaqueductal gray following a learned helplessness paradigm was associated with despair and anhedonia in rats (Ho et al. 2018); increased transcription of the glutamate receptor 1 (GluR1) in the lateral habenula following CUMS was found to regulate depressive-like behaviors (Shen et al. 2019). These critical

findings reveal the need to expand our investigation of stress-induced changes in glutamatergic signaling to other brain regions.

The complexity of changes in glutamatergic signaling in response to chronic stress is further complicated by other factors, including sex and age at stress exposure. Sex is not a variable commonly included in preclinical research despite the well-known increased risk and prevalence of stress-induced mood disorders in women. Studies that used both males and females in their design consistently reveal sex-specific effects. Exposure to CUMS increases glutamatergic transmission onto GABAergic cells in the PFC of female mice but decreases GluN2B expression only in the male PFC (Shepard and Coutellier 2018). Using the chronic social defeat stress (CSDS) paradigm, Rappeneau et al. (2016) showed that a depressive phenotype is more associated with disruption of glutamatergic transmission in the females' PFC-striatal network. These findings highlight the importance of studying the effects of chronic stress on glutamatergic signaling and emotional impairments in both males and females to capture sex-specific effects that could inform scientists when developing personalized therapeutic strategies.

Finally, age at stress exposure also plays a vital role in glutamatergic transmission. For instance, mGluR1, 2/3, and 5 are particularly sensitive to prenatal chronic stress (Lin et al. 2018b; Wang et al. 2015; Buonaguro et al. 2020), leading to decreased glutamate release in the ventral HIPP due to reduced levels of synaptic vesicle-related proteins (Marrocco et al. 2012, 2014).

Future Directions of CMS Studies

Finding safe and efficacious treatments for stress-related disorders like anxiety and depression must remain a priority for the scientific community. Exposure to stress is the leading risk factor to these psychiatric disorders providing a way to mimic their etiology in animal models. Unfortunately, stress is not a unified concept, and its effects on the brain and behaviors can vary not only by the stress procedure itself but also by the biological characteristics of the subject exposed to the stress (e.g., sex, age, estrus cycle for females, the strain of mice) (Mozhui et al. 2010; McWhirt et al. 2019). Inadequate translation from animal to human studies and FDA approval of drugs reveals significant limitations and weaknesses in our current models. The increased complexity of depression or anxiety pathophysiology is also likely to contribute to overall difficulties when translating animal trial results into humans. One way of accommodating all presented challenges, including validation of stress paradigms across sex, age, and models by scientific community members, might be achieved by adopting similar standards among research laboratories involved in preclinical animal studies on chronic stress and stress-related disorders.

3.2.3.2 Glutamate Role in Chronic Restraint Stress Animal Model

Chronic restraint stress (CRS) has been widely used to induce stress-related behavior and morphological and hormonal changes in rodent brain areas such as the HIPP, amygdala, PFC, and NAc (Kvetňanský and Mikulaj 1970; Buynitsky and Mostofsky 2009; Qiao et al. 2016). This animal model consists of keeping the animals in a cylindrical or semi-cylindrical tube with ventilation holes for 120–180 min (Padovan and Guimarães 2000; Campos et al. 2010, 2013). Seewoo et al. (2020) recently used magnetic resonance imaging (MRI) to validate this model for the study of depression. They found that rats subjected to CRS showed hypoconnectivity within the salience and interoceptive neuronal networks and hyperconnectivity of several brain regions, including the cingulate cortex. Moreover, proton magnetic resonance spectroscopy revealed reduced HIPP volume and decreased sensorimotor cortical glutamate, glutamine, and combined glutamate-glutamine levels (Seewoo et al. 2020).

Studies have used CRS to investigate the role of glutamate in certain stress-related psychiatric disorders, such as depression and anxiety. Indeed, Li et al. (2020) demonstrated that an initial increase in glutamate levels is essential in developing depressive-like behavior in the CRS model. Interestingly, Sodium Valproate (a drug that increases GABA levels in the CNS and blocks voltage-gated ion channels) and Lamotrigine (a drug that selectively binds to sodium channels, stabilizing presynaptic neuronal membranes and inhibiting glutamate release), but not Fluoxetine, reversed the decrease in dendritic spines associated with depressive behavior induced by CRS (Li et al. 2020). Liu et al. demonstrated that CRS increased anxiety in stressed mice by augmenting the prefrontal excitatory transmission from dorsomedial PFC to the BLA (Liu et al. 2020). In contrast, optogenetic stimulation of light-sensitive proteins in the neuronal cell membrane within the same pathways normalized glutamate release and reversed anxiety-like behavior induced by CRS (Liu et al. 2020).

Both acute restraint stress and CRS in mice induce anxiety-like behaviors, which are accompanied by downregulation of the ionotropic glutamatergic receptor subunits, such as NR2A subunit of NMDAR, and GluR1, GluR2 AMPAR subunits, but also by a decrease in vesicular glutamate transporters 2 (VGLUT2) concentration, in the HIPP (Zhang et al. 2019). A recent study discovered that LY487379, an mGluR2 positive allosteric modulator, reversed depressive-like behavior and electrophysiological profile changes in the dentate gyrus, occurring due to glutamatergic transmission alterations induced by CRS (Mango et al. 2019). Dygalo et al. used the same model and showed that stress-induced anhedonia, a prominent symptom of depression, manifests due to changes in expression of multiple genes involved in glutamatergic neurotransmission located in the midbrain and HIPP (Dygalo et al. 2020). Other groups also looked into the interaction between chronic ethanol use and response to CRS. For instance, Marty et al. demonstrated that chronic intermittent ethanol exposure dysregulated rat responses to CRS due to increased GluN2B subunit-dependent NMDAR function (Marty et al. 2020). Along the same lines, Carzoli et al., in their experiments, used adolescent mice, which are, as human

adolescents, more sensitive to chronic ethanol exposure. They primarily noted long-lasting changes in the postsynaptic neuroplasticity mediated by NMDAR in the bed nucleus of the stria terminalis. According to the authors, those changes due to early alcohol use may set the tone for increased stress-induced drinking "slips" during adulthood (Carzoli et al. 2019).

3.2.3.3 Glutamate Role in Chronic Social Defeat Stress Animal Model

Chronic social defeat stress (CSDS) is a standard stress model occurring in rodents and is often used in preclinical research of chronic stress and stress-related disorders (Beery and Kaufer 2015). In addition, this animal model is relevant for studying the effects of psychosocial stressors (similar to those in humans), usually associated with low socioeconomic status and a sense of powerlessness (Lowry and Jin 2020). Chronic social defeat protocol consists of placing an "*intruder*" mouse of one strain into the cage of an aggressive "*resident*" mouse of a different strain (e.g., C57BL/6 intruder vs. CD-1 resident) (Toyoda 2017). The resident mouse naturally attacks the intruder one or more times, after which the two mice are separated by a transparent screen so that visual and auditory threats can continue until the intruder is returned to its home cage. The paradigm is repeated for a week or more, typically resulting in the expression of depressive-like behavior, anhedonia, and changes in the HPA axis activity in subordinated mice (Venzala et al. 2012; Golden et al. 2011).

Mice susceptible to the above-presented protocol typically exhibit low extrasynaptic NMDARs within the HIPP (Tse et al. 2019). Along the same lines, susceptible but not resilient rats subjected to the CSDS paradigm displayed a decrease in the number of the ventral nucleus of dorsal raphe (DRv) glutamatergic neurons containing vesicular glutamate transporter type 3 (VGLUT3) in their synaptic terminals (Prakash et al. 2020). Other studies also suggested the importance of mGluRs in mediating CSDS response. For instance, knockout mice lacking mGluR2 were found to be resilient to developing corticosterone-induced escape deficits and CSDS-induced anhedonia (Highland et al. 2019). Wagner et al. (2015) investigated the role of Homer1 (a postsynaptic scaffolding protein that links mGluR5 to downstream targets) and mGluR5 in the context of the CSDS model. They demonstrated that Homer1/mGluR5 activity moderated animal vulnerability to chronic social stress. The same authors showed that CSDS-induced behavioral alterations could be partially reversed by chronic treatment with an mGluR5 inverse agonist CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazole-4-yl) ethynyl)pyridine). Finally, Jiang et al. (2020) revealed that mGluR5-dependent long-term potentiation (LTP) is involved in developing depressive-like behaviors in the mice model of CSDS-induced depression.

Another study investigated the differences between exposure to the social stressors used in the CSDS protocol and environmental stressors applied in the CMS model of chronic animal stress. The main findings were that mice exposed to CMS displayed mainly depressive-like behavior, anhedonia, and memory impairment. In contrast, those exposed to SDS showed stress-induced anhedonia,

hyperactivity, social avoidance, and anxiety (Venzala et al. 2013). In addition, the CMS model mainly disrupted excitatory-inhibitory neurotransmission balance in the PFC and brainstem while CSDS primarily reduced dopamine transmission within the same brain regions (Venzala et al. 2013). Finally, Liu et al. (2018) compared CMS and CSDS protocols through bioinformatics-driven analysis. They found that the effects of the CMS were mainly associated with disruptions in lipid and glutamate metabolism. In contrast, CSDS was more affecting cell signaling, cellular proliferation, and neuronal development, suggesting that exposure to different external stressors leads to stressor-specific neuronal molecular changes.

The most important limitation of the CSDS paradigm is that it poses significant difficulties for application in female rodents and therefore cannot be used as a universal animal model for understanding the neurobiology of human stress-related disorders, such as anxiety and major depressive disorder, as both of them are more commonly occurring in women than men.

3.2.3.4 Glutamate Role in Chronic Subordinate Colony Housing Animal Model

Chronic subordinate colony housing (CSC) is an adequate rodent model of chronic psychosocial stress. Animals exposed to this model have reduced body weight gain, increased adrenal weight and corticosterone levels, intestinal immune activation, long-lasting anxiety-like behavior (Nyuyki et al. 2012; Langgartner et al. 2015), and PTSD-like symptoms (Reber et al. 2016). This animal model mimics the type of health-compromising and disease-promoting stressors that humans potentially face every day. It is based on the innate drive of male mice to establish a hierarchical order within their colony. The largest male will emerge as dominant and force the remaining mice into a subordinate position by repeated aggressive attacks and threats, especially during the first hour of establishing social hierarchies (Reber and Neumann 2008; Masis-Calvo et al. 2018). Data available in the literature, although still scarce, point to the role of glutamatergic neurotransmission in this model of chronic social stress (Peterlik et al. 2015). Indeed, Peterlik et al. (2017b) used CSC for 19 days to investigate whether central mGluR7 is altered upon chronic psychosocial stressor exposure and if genetic ablation of mGluR7 interferes with the multitude of chronic stress-induced alterations. The primary study finding was that CSC induced downregulation of mGluR7 mRNA expression in the PFC. When the same protocol was applied to the mGluR7 deficient mice, they were less vulnerable to CSC-induced stress, evidenced by their reduced physiological and immunological responses (Peterlik et al. 2017b). In addition, Peterlik et al. reported on mGluR5 role in mediating chronic psychosocial stress consequences in mice (Peterlik et al. 2017a). They demonstrated that mGluR5-deficient mice expressed less vulnerability to the CSC protocol (Peterlik et al. 2017a). They also showed that chronic treatment of mice with CTEP, an inverse mGluR5 agonist, prevented the occurrence of adverse hormonal and immunological consequences caused by CSC (Peterlik et al. 2017a). Finally, they suggested the inverse agonism of CTEP on CSC-induced upregulation

of mGluR5 in the HIPP as a potential mechanism relevant for its stress-protective effects (Peterlik et al. 2017a).

3.2.3.5 Glutamate Role in Rat Cumulative Allostatic Load Measure Animal Model

Stressful situations experienced throughout life impact the physiology of the metabolic, immune, and neuroendocrine systems (McEwen and Stellar 1993). Once the stress starts to become chronic or recurrent, it is even more difficult for an organism to maintain allostasis and reduce "*allostatic load*" (AL) resulting from repeated environmental challenges (McEwen and Stellar 1993; McCreary et al. 2019). To assess the burden of chronic stress in preclinical animal translational research more precisely, McCreary et. (2019) recently introduced the "*rat cumulative allostatic load measure*" (rCALM). The new translational assessment contains twelve biomarkers most commonly related to stress physiology and behavior noted in rat and mouse preclinical research studies. Interestingly, the authors suggested that rCALM might also serve as a valuable tool for measuring the therapeutic benefit of lifestyle interventions aiming to reduce AL (McCreary et al. 2019).

The role of glutamate neurotransmission in AL has recently gained a lot of attention among scientific community members. Coplan et al. studied the impact of maternal response to AL during infant rearing on the neurobiological measures of the grown offspring. They found that mothers exposed to allostatic overload during infant rearing showed increased levels of CRF in cerebrospinal fluid (CSF), which was correlated with a proportional increase of CSF glutamate levels. Moreover, high maternal CSF glutamate levels were associated with serotonin-metabolism alterations in young adolescent offspring, manifested as a persistent increase in CSF 5-hydroxyindoleacetic acid (5-HIAA) concentrations (Coplan et al. 2018).

3.2.4 Glutamate Role in Oxidative and Nitrosative Cellular Stress

Under normal physiological conditions, glutamate is essential in regulating and modulating memory, learning, mood, and neuroplasticity. However, when in excess, it is associated with neurotoxicity resulting in pathological processes manifested as deficits in cognition and decreased mood. Noteworthy, glutamate-induced neurotoxicity occurs due to extracellular glutamate accumulation, causing an increase of intracellular Ca^{2+}, resulting in the excessive production of reactive oxygen species (ROS) generated by mitochondrial dysfunction and reduction of cellular antioxidant capacity (Schinder et al. 1996; Stanciu et al. 2000).

ROS and reactive nitrogen species (RNS) act as messengers, regulating pathways involved in cell survival and cell death. ROS and RNS are continuously removed by

endogenous antioxidant enzymes, such as glutathione, catalase, and superoxide dismutase (Locatelli et al. 2003). When ROS and RNS are present in excess in the cell, their concentrations exceed the cell's antioxidant capacity and lead to a condition called oxidative stress (Sies 1997). Oxidative stress is associated with microglial-mediated inflammation and the release of inflammatory mediators (Leszek et al. 2016). This kind of microglial activation results in an oxidative burst, which releases ROS, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), the highly reactive hydroxyl radical (HO), and RNS such as nitric oxide (NO), which could also be increased through NMDAR activated NO synthesis (Tufekci et al. 2012; Garthwaite et al. 1989). Activated microglia and monocyte-derived macrophages can induce glutamate excitotoxicity by extruding glutamate into the extrasynaptic space in exchange for cystine via the cystine/glutamate exchanger (Xc)–transporter (Kigerl et al. 2012) (see Fig. 3.1). Finally, the enhanced immune response increases glutamate-like molecule quinolinic acid, which over-excites astrocyte's NMDARs, restrains glutamine synthetase (GS), a critical enzyme in the glutamate-glutamine cycle in astrocytes keeping the stable glutamate level, thus promoting glutamate release into extracellular space (Guillemin 2012; Chen et al. 2019b).

In neurodegenerative diseases such as AD, oxidative stress is associated with cellular death mediated by the glutamatergic system (Findley et al. 2019). Indeed, soluble amyloid-beta (Aβ) stimulates an excessive ROS generation from continuously activated NMDAR, leading to oxidative damage and synaptic impairment (De Felice et al. 2007; Shelat et al. 2008). Furthermore, the increased production of ROS and RNS and the reduction of antioxidant substances lead to neuronal cell death in AD (Farooqui and Farooqui 2009; Melo et al. 2011).

Similarly, PD pathogenesis is associated with oxidative stress, inflammation, and gut dysbiosis (Chen et al. 2019b). Moreover, rats exposed to the CMS protocol, an animal model of chronic stress, combined with the 6-hydroxydopamine (6-OHDA) model of PD, showed depressive-like behavior along with oxidative damage and reduced antioxidant capacity in the PFC, HIPP, and striatum (Tuon et al. 2021).

Glutamatergic modulators are also capable of influencing oxidative stress parameters. Ketamine, for instance, potentiates oxidative stress and influences schizophrenia-like behaviors and inflammation in response to lipopolysaccharide (LPS) exposure in early life (Réus et al. 2017). However, when administered in subanesthetic doses associated with antidepressant-like effects, Ketamine seems to exert antioxidant properties. For example, in a study by Réus et al. (2015b), a single dose of Ketamine reversed neuronal changes in adult rats caused by oxidative stress due to early-life stressor exposure (maternal deprivation). When combined with electroconvulsive therapy (ECT), Ketamine protected rats against oxidative damage and the immunological response induced by ECT (Gonçalves et al. 2021). Another NMDA antagonist, Memantine, exerted similar antidepressant properties, restored increased neuronal nitric oxide synthase (nNOS) expression, NO levels, and superoxide dismutase (SOD) activity, decreased mitochondrial enzymes activity induced by chronic stress, and upregulated stress-responsive BDNF signaling (Mishra et al. 2021) (see Fig. 3.1).

3.2.5 *Glutamate Interactions with Other Neurotransmitters Relevant to Stress Response*

3.2.5.1 Gamma-Aminobutyric Acid

Changes in glutamate as the primary excitatory and gamma-aminobutyric acid (GABA) as the central inhibitory neurotransmitter metabolism may significantly affect cortical excitability (Petroff 2002). Moreover, glutamate is the metabolic precursor of GABA, which can be recycled through the tricarboxylic acid cycle to synthesize glutamate (Petroff 2002; Bak et al. 2006).

In contrast to the mature brain, GABA shows excitatory properties in the developing brain, leading to cell membrane depolarization, increasing cytoplasmic calcium, and triggering the action potential. In young neurons, glutamate can inhibit the excitatory actions of GABA at both presynaptic and postsynaptic sites, so there is no runaway excitation in the developing brain (Kendell et al. 2005).

Interestingly, the interaction between GABA and glutamate in a mature brain is varying between brain regions. For example, the AMPA and NMDA-receptor agonist infusions increased extracellular concentrations of GABA in the striatum and the NAc (Mora et al. 2008). However, MK-801 (an NMDAR antagonist) decreased the activity of GABAergic interneurons, resulting in an increase in pyramidal neuron excitability in the PFC (Cohen et al. 2015).

Finally, GABA and glutamate imbalances are highly contributing to the development of several major mental illnesses, including autism spectrum disorders (ASD) (El-Ansary and Al-Ayadhi 2014), MDD (Kendell et al. 2005), epilepsy (Bozzi et al. 2018), and schizophrenia (Carlsson et al. 2001).

3.2.5.2 Corticosteroids

The secretion of glucocorticoids (cortisol in humans and corticosterone in rodents) is a typical endocrine response to stress-induced HPA axis activation (Sapolsky et al. 2000). As reviewed by Popoli et al. (2012), acute and chronic stressors trigger the release of glucocorticoids, affecting glutamate neurotransmission in the PFC and the HIPP in region-specific ways. For example, acute stress or corticosterone treatment increased AMPAR and NMDAR responses to a similar extent in the PFC. In contrast, they selectively enhanced AMPAR-mediated currents in CA1 neurons of the HIPP. Glucocorticoids might also be responsible for mediating the effects of stress on EAAT2 regulation (Popoli et al. 2012). A preclinical study by Zschocke et al. (2005) showed that synthetic (dexamethasone) and natural glucocorticoids promote glutamate uptake in cortical astrocytes by activating the transcription and subsequent translation of the glial glutamate transporter-1 (GLT-1) gene (Zschocke et al. 2005).

Several studies reported on the effects of glutamate on corticosteroid levels. Indeed, Réus et al. (2012) demonstrated that Memantine normalized increased

corticosterone levels induced by CMS. Ketamine showed similar effects and reversed corticosterone-induced adverse changes, such as depressive-like behavior and dysregulated neurotransmission in the DRN (Sowa et al. 2019; Camargo et al. 2020; Koike et al. 2013).

3.2.5.3 Serotonin

Serotonin (5-hydroxytryptamine) is a neurotransmitter that modulates various physiological functions in the brain (e.g., stress response, food intake, mood, pain, sleep-wakefulness, etc.) and is implicated in the etiology of several mental illnesses, including stress-related diseases such as MDD and anxiety disorders (Sodhi and Sanders-Bush 2004; Hamon and Blier 2013; O'Mahony et al. 2015).

The link between serotonin and depression has been described for the first time during the 1960s under the name "*serotonin hypothesis*" (Cowen and Browning 2015). This hypothesis initially proposed that deregulation of serotonin pathways and low levels of serotonin were the leading causes of depression (Cowen and Browning 2015). "*Tryptophan depletion*" studies further supported this assumption by showing mood alterations in certain people when the serotonin precursor, amino acid tryptophan, is reduced (Cowen and Browning 2015). Practical application of this theory in pharmaceutical drug development led to the discovery of selective serotonin reuptake inhibitors (SSRIs), currently the most prescribed class of antidepressants.

Low 5-HT1B receptor binding in limbic brain regions is a typical finding noted in patients with MDD. A recent positron emission tomography (PET) study conducted by Tiger et al. (2020) demonstrated that the reduction of depressive symptoms after Ketamine treatment correlated inversely with the level of 5-HT1B receptor binding in the ventral striatum at the baseline visit. An increased (although not statistically significant) number of 5-HT1B receptors (expressed by increased 5-HT1B receptor binding) in HIPP of 30 patients with SSRI-resistant depression compared to those on placebo was also observed (Tiger et al. 2020). Other studies also suggested the importance of 5-HT1B receptors in Ketamine antidepressant mechanism of action (Murrough et al. 2011; Tiger et al. 2016; Yamamoto et al. 2013).

In their recent review of Ketamine preclinical studies, Pham and Gardier suggested another mechanism leading to increased serotonin concentrations in the synaptic cleft, occurring through serotonin reuptake inhibition. Indeed, they noted that Ketamine binds to the selective 5-HT transporter (SERT), although with a much weaker affinity when compared with the SSRIs. According to the same authors, Ketamine also shows an affinity to other serotonergic receptors, including 5-HT1, 5-HT2, 5-HT3, and 5-HT7 (Pham and Gardier 2019).

Wang et al. (2019) reported about the Dual Serotonin-Glutamate Neurons within the DRN providing significant input to VTA, a region widely implicated in the brain's drug and natural reward circuitry. These serotonergic neurons also coexpressed vesicular glutamate transporter 3 (a transporter responsible for the accumulation of glutamate into synaptic vesicles), suggesting their role in increasing

mesoaccumbenal dopamine, thus affecting various reward-related processes. Several authors demonstrated that 5-HT2A receptors and mGluR2 could also assemble into a functional heteromeric complex and modulate each other's functions which might be a potential mechanism mediating the effects of hallucinogens and certain antipsychotics (Shah and González-Maeso 2019; González-Maeso et al. 2008).

Two tryptophan catabolites, kynurenic and quinolinic acid, released via the "*kynurenine*" metabolic pathway, were noted to influence the NMDAR activity (e.g., kynurenic acid is an NMDAR antagonist while quinolinic acid acts as an NMDAR agonist with potential depressogenic properties) (O'Mahony et al. 2015). Moreover, increased quinolinic acid levels led to elevated concentrations of glutamate in the striatum and the cortex (Müller and Schwarz 2007).

3.2.5.4 Brain-Derived Neurotrophic Factor

Brain-derived neurotrophic factor (BDNF) is one of the most studied brain neurotrophins, significantly impacting neuronal survival, growth, synapse plasticity, and neurotransmitter release (Giacobbo et al. 2019; Bramham and Messaoudi 2005).

It is synthesized, stored, and released by glutamatergic neurons. BDNF binds to tyrosine kinase receptors (TrkB), potentiating excitatory synapses via pre-and post-synaptic mechanisms and increasing glutamate release (Bramham and Messaoudi 2005). In addition, it induces the expression of genes coding the synthesis of various regulators of synaptic activity and synaptic vesicle proteins, such as vesicular glutamate transporters (Leal et al. 2014).

The most significant role of the BDNF is to protect neurons against glutamate excitotoxicity (Almeida et al. 2005). Although the exact mechanism of this physiological function is still not clear, Gaidin et al. demonstrated that BDNF induced neuroprotective effects on the neurons and astrocytes by inhibiting Ca^{2+} intracellular signaling and reducing proapoptotic proteins and inflammatory cytokine expression (Gaidin et al. 2020). Antioxidant defense system (Mattson et al. 1995) and ERK (extracellular-signal-regulated kinase) and PI3-K (phosphatidylinositol 3-kinase) signaling pathways have been reported as BDNF's downstream cellular cascades (Almeida et al. 2005).

The interplay between BDNF and drugs with NMDAR antagonistic properties has been recently established. Indeed, Ketamine increased the phosphorylation (activation) of HIPP TrkB and induced a rapid increase in total BDNF protein levels (Zanos and Gould 2018). However, Ketamine failed to exert antidepressant actions in mice with BDNF gene knockdown, suggesting that BDNF signaling is necessary for its antidepressant actions (Autry et al. 2011; Lepack et al. 2015). Administration of another NMDAR antagonist, Memantine, also increased BDNF levels in the PFC of chronically stressed rats (Réus et al. 2012).

The interaction between chronic stress, glutamate, and BDNF is most evident in major psychiatric and neurodegenerative disorders. Indeed, environmental factors such as chronic stress (Esch et al. 2002; Hammen 2005), glutamate-induced neurotoxicity (Sanacora et al. 2012; Lewerenz and Maher 2015), and decreased BDNF

expression in the HIPP and PFC are involved in their initiation and progression (Giacobbo et al. 2019; Bath et al. 2013; Duman and Monteggia 2006).

3.2.6 *Stress-Induced Changes in Gene Expression and Epigenetic Modifications of the Glutamatergic System*

The role of stress in gene expression and epigenetic modifications is well established (McEwen et al. 2015). Moreover, epigenetic changes such as DNA methylation, histone modification, and nucleosome positioning explain how stressors interact with the genome, leading to alterations in DNA structure, gene expression, and behavioral patterns (Turecki and Meaney 2016; Park et al. 2019; Portela and Esteller 2010).

Several authors reported on the interactions between stress, glutamatergic neurotransmission, and gene expression. For instance, two research groups demonstrated the impact of prenatal restraint stress on reducing glutamate release in the ventral HIPP of laboratory animals. The changes presented reduced expression and functionality of mGluR1 and mGluR5 in males and mGluR 2/3 in males and females (Zuena et al. 2008; Maccari et al. 2014). Another study showed that prenatal restraint stress (PRS) decreased mGluR2 and mGluR3 receptor mRNA and protein levels in the FC, manifested at birth and persisting in adulthood. Nasca et al. reported that the same type of stress decreased the gene transcription of the presynaptic mGluR2 receptor (which, once activated, reduces glutamate release) in the HIPP (Nasca et al. 2015a).

Evidence about the interaction between stress, glutamate neurotransmission, and epigenetic modifications was also provided by Matrisciano et al. (2018), who found that mice subjected to PRS showed epigenetic changes in mGluR2/3 (Matrisciano et al. 2018), while Cao et al. (2016) demonstrated that stress-induced histone modification modulated mGluR2/3 expression in the spinal cord (Cao et al. 2016).

3.2.7 *Stress Effects on Microglia–Glutamate Interactions*

Microglia play a pivotal role in the normal development and regulation of structural and functional processes of the brain, from individual synapses to neural circuits and behaviors. They are responsible for eliminating microbes, dead cells, redundant synapses, protein aggregates, and other particulate and soluble antigens that may endanger the brain (Reus et al. 2017; Colonna and Butovsky 2017; Helmut et al. 2011; Schafer and Stevens 2015). Microglia also exhibits neuroprotective effects mediated by stimulation of neurotrophin release, glutamate uptake, and sequestering of neurotoxic substances (Colonna and Butovsky 2017; Block and Hong 2005). In

contrast, uncontrolled and over-activated microglia typically reacts by excessive release of pro-inflammatory substances resulting in neuroinflammation and neurotoxicity (Colonna and Butovsky 2017; Block et al. 2007).

Various external and internal stressors might activate and sensitize microglia to increased immunological reactivity (Frank et al. 2007). Tynan et al. (2010) demonstrated that chronic stress altered the density and morphology of microglia in a subset of stress-responsive brain regions (Tynan et al. 2010). Takatsuru et al. showed that exposure to early-life stress increases microglia motility in adulthood (Takatsuru et al. 2015).

While in a resting or *"surveillance state"*, microglia express neither glutamate receptors nor transporters. In contrast, upon activation, microglia release large amounts of glutamate (Barger et al. 2007), with glutamate receptors and transporters appearing on their surfaces (Haroon et al. 2017). Microglial cells express both iGluR and mGluR. Moreover, activation of a particular glutamate receptor is followed by a release of a specific cytokine. For instance, potentiation of AMPAR transmission inhibited the further release of tumor necrosis factor-alpha (TNF-α) from microglial cells. In contrast, activation of mGluR2 showed the opposite effects resulting in increased microglia-mediated neurotoxicity (Colonna and Butovsky 2017; Helmut et al. 2011). Takeuchi et al. demonstrated that cytokine TNF-α could also directly induce excessive glutamate release from activated microglia in an autocrine signaling manner by upregulating microglial glutaminase (the enzyme that converts glutamine to glutamate) (Takeuchi et al. 2006). Finally, agonists of group three (III) mGluR receptors reduced microglial reactivity, suggesting that activation of these receptors can protect neurons against microglia-mediated neurotoxicity (Taylor et al. 2003).

Acknowledgments Translational Psychiatry Laboratory (Brazil) is supported by grants from CNPq (GZR), FAPESC (GZR), CAPES (GZR), Instituto Cérebro e Mente (GZR), and UNESC (GZR). GZR is a 2 CNPq Research Fellow. LMM is a CNPq Research Fellow.

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Suggested Reading

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Chapter 4
Current Glutamatergic Treatments and Future Directions for Glutamate-Based Management of Chronic Stress and Stress-Related Disorders

Laurence D. Coutellier, Laura Musazzi, and Zoran M. Pavlovic

Abstract The recent Food and Drug Administration and European Medicine Agency regulatory approval of intranasal (S)-Ketamine for the treatment-resistant depression in adults and its quick introduction in everyday psychiatric practice might be considered as a second revolution in the field of neuropsychopharmacology after the first one, which started over 50 years ago with the discovery of the monoaminergic antidepressants. This chapter will present the critical antidepressant features of Ketamine, including its putative mechanisms of action and other Ketamine-like glutamatergic drugs in development for the treatment of chronic stress-related psychiatric disorders. First, a detailed picture of NMDA-dependent and independent glutamatergic mechanisms mediating Ketamine effects will be given. The following section will be dedicated to an in-depth description of cellular and molecular mechanisms which have been suggested to underlie the fast antidepressant properties of Ketamine. Alternative investigational therapeutic uses of Ketamine (besides as an antidepressant) will then be presented, followed by the description of other glutamatergic drugs which received significant attention for their potential antidepressant effects.

The following part will be dedicated to limitations related to Ketamine use. Finally, we will share our insights and provide recommendations on the future perspectives in the prevention and early management of chronic stress and stress-related disorders, focusing on glutamate-based aspects of proposed interventions.

Keywords Chronic stress prevention · Epigenetics · Nutritional supplements · Biomarkers in stress-related disorders · Ketamine-like substances

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_4

4.1 Current Glutamatergic Treatments for Chronic Stress and Stress-Related Disorders

Major depressive disorder (MDD) has been historically treated with monoaminergic antidepressants that increase monoaminergic neurotransmission in the human brain. This approach has been moderately effective in helping depressed patients: first, these compounds need several weeks to improve the depressive symptoms. Then, many patients are considered treatment-resistant. Last, these drugs are also associated with frequent side effects (Neis et al. 2016). These findings strongly point out an urgent need to develop fast-acting antidepressants with better efficacy and fewer side effects.

Along the same lines, during the last few decades, the scientific community's interests regarding the potential antidepressant properties of the anesthetic drug Ketamine, an N-methyl-D-aspartate receptor (NMDAR) antagonist, showed significant growth. Ketamine is well-known as one of the safest anesthetic agents, approved by the FDA for anesthetic use in 1970, exhibiting NMDAR antagonistic properties reported for the first time in 1983 (Wei et al. 2020a). It was noted that the anesthetic effects of Ketamine are driven mainly by disrupting connectivity between and within resting-state consciousness networks, particularly frontoparietal connectivity (Bonhomme et al. 2016). The first report of the antidepressant action of (*R, S*)-Ketamine in animal models was published in 1975 (Sofia and Harakal 1975). But this is not before 2000 that its antidepressant effect was also confirmed in human studies (Berman et al. 2000), together with its dissociative and psychotomimetic adverse events and risk for abuse (Shin and Kim 2020).

Ketamine improves depressive symptoms within 24 h after receiving the first dose, helps patients that are otherwise treatment-resistant (Murrough et al. 2013), and has long-lasting properties (efficacy over 15 days) (Singh et al. 2016). Current research aims at fully understanding the mechanisms of action of Ketamine to potentially develop novel compounds that would be similarly fast-acting and long-lasting but without the side effects.

Still, our current knowledge on the Ketamine mechanism of action leading to its fast and significant reduction in depressive symptoms is modest and is mainly derived from rodent models of depression. These preclinical paradigms are typically based on exposure to stressful events since stress-induced effects on neural plasticity and memory are critical for depression pathogenesis (Wang et al. 2015). Moreover, exposure to chronic unpredictable mild stress, chronic social defeat stress, or the development of learned helplessness leads to structural, neuronal, physiological, and molecular changes in the rodent brain that are reminiscent of what is observed in the brain of depressed patients. These changes include prefrontal and hippocampal (HIPP) volume reduction, HPA axis dysregulation, inflammation, oxidative stress, neurotransmitter disturbances (Belleau et al. 2019; Khan et al. 2020a), and large-scale functional alterations such as network-wide glutamate functional hyperconnectivity (McGirr et al. 2017).

Antidepressant properties of candidate compounds such as Ketamine are typically tested in behavioral assays that are believed to inform on specific behavioral abnormalities seen in MDD. These animal models include the forced swim test (FST) as a measure of despair, the novelty-suppressed feeding (NSF) test as a measure of anxiety (a common comorbid condition to MDD), and the sucrose preference test (SPT) as a measure of anhedonia. Noteworthy, FST is sensitive to the protracted effects of Ketamine up to 1 week after an acute injection, the anxiety level is reduced in the NSF test after a single dose of Ketamine, and chronic stress-induced anhedonic behaviors are usually rescued by Ketamine up to 8 days after the injection (Browne and Lucki 2013). Others have shown that Ketamine can also promote cognitive function in rodent chronic stress models of depression, with a single subanesthetic dose of Ketamine restoring cognitive flexibility by enhancing plasticity in the ventral HIPP-PFC pathway (Jett et al. 2015). While the gap between preclinical models and clinical situations exists and is well recognized by the scientific community, those models are necessary to decipher the mechanisms of action of potential antidepressant compounds and inform future directions that should be taken to develop drugs that are safe and effective.

4.1.1 Mechanisms of Action of Ketamine in Chronic Stress

4.1.1.1 Ketamine Effects on NMDAR-Independent Glutamate Neurotransmission

Although Ketamine has been shown to act as an antagonist of the GluN2B subunit of the NMDAR (Jie et al. 2018), several studies have demonstrated that its antidepressant effects also require the activation of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) (Aleksandrova et al. 2017; Chou and Peng 2018; Duman et al. 2019; Elhussiny et al. 2021; Yang et al. 2018a). This finding led to the hypothesis that the initial increase in synaptic glutamate neurotransmission following Ketamine administration preferentially activates AMPARs, promoting synthesis and release of brain-derived neurotrophic factor (BDNF). The contribution of AMPARs to Ketamine antidepressant properties has been further supported in a study by Gilbert et al. who used magnetoencephalography (MEG is a functional neuroimaging technique for mapping brain activity by recording magnetic fields produced by electrical currents occurring naturally in the brain, using very sensitive magnetometers) analysis together with dynamic causal modeling (an approach that uses a biologically informed model to make inferences about the activity of neuronal networks generating MEG responses) to investigate AMPAR- and NMDAR-mediated connectivity changes following Ketamine administration in subjects with treatment-resistant MDD and healthy controls. Their main finding was that the effects of the Ketamine-induced AMPAR neurotransmission lasted much longer (i.e., up to 11 days post-infusion) than those achieved via NMDAR receptors and suggested that AMPAR activation might be critical in mediating Ketamine long-term antidepressant effects (Gilbert et al. 2018).

Several researchers have also discussed the importance of the mGluR5 signaling during the initial rise in glutamate levels following Ketamine administration. Ketamine reduced mGluR5 availability via a rapid receptor internalization mechanism for at least 24 h post-treatment in vivo in humans, which correlated with its antidepressant properties (Delorenzo et al. 2015; Esterlis et al. 2018a). These findings appear counterintuitive as rodent models of MDD and postmortem human studies report decreased mGluR5 expression (Shin et al. 2015), and mice lacking this receptor display depressive-like behaviors and failed to induce delta-FosB expression in the nucleus accumbens (NAc), a marker of stress resilience (Esterlis et al. 2018b). But due to the close localization between mGluR5 and NMDAR on the cell surface, drugs that target mGluR5 may indirectly bring about a therapeutic effect through NMDAR modulation (Esterlis et al. 2018b).

4.1.1.2 Ketamine Effects on Gamma Aminobutyric Acid Neurotransmission

Antagonism of NMDAR is necessary for inducing a transient surge in presynaptic glutamate release resulting in increased cortical pyramidal cell excitation within the PFC, a brain region playing a critical role in emotion regulation (Abdallah et al. 2018a). Along the same lines, a pilot proton magnetic resonance spectroscopy in depressed patients showed that in addition to an acute increase in Glx (combined glutamate + glutamine measure), Ketamine increased gamma aminobutyric acid (GABA) levels in the medial PFC (mPFC) (Milak et al. 2016). However, this preliminary finding was not replicated in a follow-up study (Milak et al. 2020).

Further investigations supported these initial findings. For instance, magnetic resonance spectroscopic imaging (MRSI is a noninvasive imaging method that provides spectroscopic information which could be used to infer further information about cellular activity such as metabolic information) in healthy human subjects showed that Ketamine decreased HIPP GABA+/total Creatinine ratio 2 h post-infusion, without changing Glx (glutamate + glutamine) levels, meaning that Ketamine effects on GABA transmission were not mediated via glutamatergic mechanisms (Silberbauer et al. 2020). In the same way, in rodent models, Ketamine induced an acute, transient suppression of prefrontal GABAergic neurons through antagonism at the GluN2B subunit of NMDAR (Li et al. 2011; Gerhard et al. 2020), restoring the excitatory/inhibitory balance altered by chronic stress exposure (Ghosal et al. 2017, 2020) (see Fig. 4.1).

Specific sub-population of GABA neurons in the PFC might drive the rapid antidepressant effects of Ketamine, such as somatostatin- and parvalbumin-expressing interneurons (Gerhard et al. 2020). Ketamine increases the activity of parvalbumin-expressing GABA interneurons sufficiently to protect dendritic spines against the effects of stress (Ng et al. 2018). Moreover, targeting NMDAR of fast-spiking GABA interneurons by Ketamine was noted in the HIPP of adult male rats, leading to disinhibition of pyramidal cells. Interestingly, the exact mechanism was reported for Rapastinel (a glycine site NMDAR partial agonist) and Scopolamine

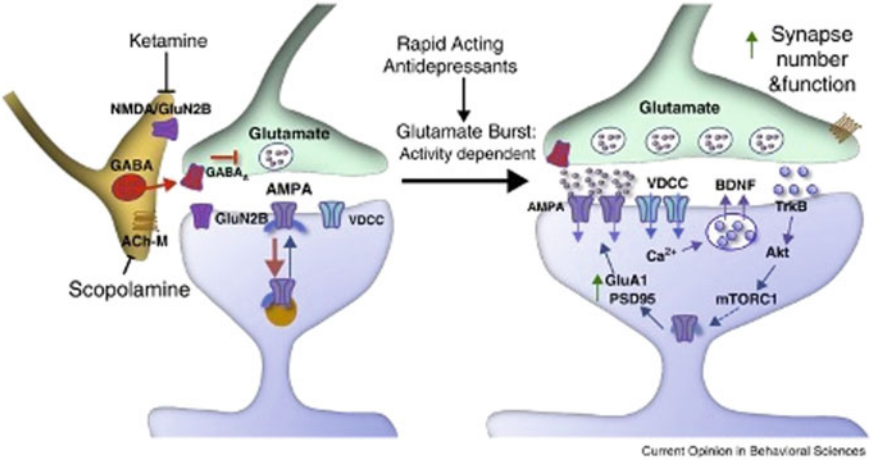


Fig. 4.1 Schematic representation of ketamine-mediated disinhibition of mPFC pyramidal neurons via inhibition of GABAergic interneurons (GIs) underlying Ketamine Disinhibition Hypothesis. Ketamine triggers a burst of glutamate that is thought to occur via inhibition of the GIs (primarily those from the low threshold spiking group with the highest basal firing rates); NMDARs drive the tonic firing of these GIs, and the active, open-channel state allows Ketamine to enter and block NMDA channel activity of the interneurons. This results in Ketamine-induced glutamate burst stimulating AMPARs, which causes depolarization and activation of voltage-dependent Ca^{2+} channels (VDCC), leading to release of BDNF and stimulation of TrkB and Akt pathway, which then activates mTORC1 signaling, leading to the increased synthesis of proteins that are required for synapse maturation and formation (i.e., GluA1 and PSD95) (from Gosal et al. 2017)

(an antagonist of the muscarinic acetylcholine receptor) (Widman and McMahon 2018).

It is worth mentioning that changes in PFC and HIPP interneurons activity by Ketamine directly affect the overall activity of brain circuits and likely restore the balance between excitation and inhibition that is often impaired in MDD and rodent models of depression. This disinhibition hypothesis of Ketamine explains the burst of glutamate observed, which triggers the second phase stimulating AMPARs, which causes depolarization and activation of voltage-dependent Ca^{2+} channels (VDCC), inducing release of BDNF and stimulation of TrkB and Akt pathway, which then activates mTORC1 signaling, leading to the increased synthesis of proteins that are required for synapse maturation and formation, necessary for the antidepressant effects of Ketamine.

4.1.1.3 Ketamine Effects on Dopamine Neurotransmission

Other researchers have emphasized the potential modulation of dopaminergic transmission by Ketamine. Anhedonia and despair, two symptoms of MDD, are attributed to abnormal functioning of the ventral tegmental area (VTA)-nucleus accumbens (NAc) pathway. Addiction, an often comorbid condition in MDD, is also associated with abnormal functioning of the NAc, which is also found to be

altered in rodent models of depression, including in the social defeat stress paradigm (Xu et al. 2020). In rodent models, rats exposed to chronic stress have less active dopaminergic (DA) cells in the VTA (Rincón-Cortés and Grace 2017, 2020) and displayed NAc hypertrophy (Abdallah et al. 2017), findings similar to those observed in patients with MDD [656]. Ketamine increased the number of active DA cells in the VTA of rats (Rincón-Cortés and Grace 2017, 2020) and moderately reduced NAc volume in MDD patients (Abdallah et al. 2017). These data strongly indicate that some of the antidepressant effects of Ketamine might be driven via changes in dopaminergic transmission, at the level of VTA-dopamine neurons and through D2 and D3, but not D1 receptors (Blum et al. 2019; Chang et al. 2020), leading to an increase in extracellular dopamine in the NAc (Witkin et al. 2016). Imaging studies in remitted, treatment-free depressed patients strongly support a significant effect of Ketamine on brain regions involved in reward response (such as NAc) at 2 h post-infusion, an effect independent of changes in depressive symptoms (Kotoula et al. 2020).

The above findings contradict other studies that failed to show Ketamine direct effects on mesolimbic DA neurotransmission. For instance, Can et al.'s study in mice reported that Ketamine did not alter the magnitude or kinetics of evoked DA release in the NAc, and concluded that in vivo changes in DA neurotransmission following Ketamine administration observed in similar studies are likely indirect (Can et al. 2016). Another preclinical research study in mice showed that one day after injection, Ketamine and its metabolite (2R,6R)-hydroxynorketamine (HNK) altered the function of AMPARs and synaptic plasticity in VTA-NAc circuits. Ketamine also induced long-term depression in VTA-dopaminergic neurons, which might lead to decreased dopaminergic modulation of the NAc (Yao et al. 2018). The discrepancy in results between these two and other previously mentioned animal studies might be at least partially explained by differences in Ketamine concentrations or by the fact that the last two preclinical experiments administered Ketamine to non-stressed animals rather than those exposed to chronic stress animal paradigms already showing deficiencies in dopaminergic neurotransmission within reward-related neuronal circuits. These preclinical findings perfectly translate to those from human studies as several clinical trials demonstrated the opposite effects of acute administration of Ketamine on healthy people and those observed in patients with MDD.

In addition to restoring anhedonic and reward-related behaviors in depressed subjects by increasing activity of the VTA-dopaminergic neurons, Ketamine could also contribute to rescuing other behavioral domains (e.g., aversive learning) by directly modulating synaptic structural plasticity in the PFC (Wu et al. 2020). Indeed, others reported an increase in dopamine levels in different brain regions after acute Ketamine administration in rodents, including the cortex and striatum (Kokkinou et al. 2018). For instance, Lally et al. demonstrated that Ketamine induced an increase in regional cerebral metabolic rate of glucose (rCMRGlu), a marker of glucose metabolism) in the dorsal anterior cingulate cortex (dACC) of depressed patients (Lally et al. 2015). This mechanism might serve as an explanation of Ketamine ability to reduce anhedonia rapidly.

4.1.1.4 Ketamine Effects on 5-HT Neurotransmission

The impact of Ketamine on 5-HT (serotonin) neurotransmission has also attracted neuroscientists' attention. Several studies showed that Ketamine causes a robust increase in extracellular serotonin in brain regions regulating emotions, including the PFC and the HIPP. Interestingly, the mechanisms leading to increased serotonergic neurotransmission vary between the two Ketamine enantiomers. In a study by Ago et al. (R)-Ketamine strongly activated the prefrontal serotonergic system through AMPA receptor-independent mechanism, while (S)-Ketamine induced serotonin and dopamine release in an AMPA receptor-dependent way (Ago et al. 2019). Sowa et al. demonstrated that a single dose of Ketamine restored a chronic corticosterone-induced imbalance between the excitatory and inhibitory neurotransmission within the dorsal raphe nucleus (DRN) of rats, the primary source of 5-HT forebrain projections (Sowa et al. 2019).

Others reported that Ketamine increased serotonin release in the PFC of control mice via stimulation of prefrontal AMPARs (Nishitani et al. 2014; Araki et al. 2014). Fukumoto et al. suggested that the activation of serotonergic neurons through a similar mechanism in the DRN might play a critical role in Ketamine antidepressant effects (Fukumoto et al. 2016). This finding is further supported by experiments showing that in 5-HT depleted rats, the effects of Ketamine on stress-induced immobility in the forced swim test are significantly diminished (Gigliucci et al. 2013). Recent work suggests that Ketamine additionally inhibits 5-HT clearance in the HIPP via a serotonin transporter (SERT)- and plasma membrane monoamine transporter-(PMAT) dependent mechanism. Indeed, studies in SERT and PMAT knockout mice showed the absence of Ketamine antidepressant effects (Bowman et al. 2020). Other researchers emphasized that stimulation of a 5-HT1A receptor in the PFC and subsequent activation of the Akt/mTORC1 signaling also contributes to the sustained antidepressant effects of Ketamine (Fukumoto et al. 2018).

Another research avenue pursued to elucidate the role of 5-HT in Ketamine antidepressant effects focuses on tryptophan (Trp) metabolism. Interestingly, in two rodent models of depression, Ketamine administration did not affect quinolinic acid plasma concentrations (a metabolite produced via the kynurenine metabolic pathway, found to be increased in depressed patients), while a chronic Fluoxetine administration decreased quinolinic acid plasma levels (Eskelund et al. 2017). In contrast, a pharmacometabolomics study by Rotroff et al. in patients with TRD showed that both Ketamine and (S)-Ketamine altered metabolites related to tryptophan metabolism (for example, indole-3-acetate and methionine). Interestingly, the only metabolic signature associated with response to treatment was kynurenine (KYN)/tryptophan (TRP) ratio, indicating that the amount of TRP was increased relative to KYN in subjects with a more significant decrease in depressive symptoms (Rotroff et al. 2016). These findings were further supported by Moaddel et al. who demonstrated that MDD patients responding to Ketamine present lower KYN levels and KYN/TRP ratios than nonresponders and suggested these two metabolic parameters as potential biomarkers predicting response to Ketamine (Moaddel et al. 2018).

4.1.1.5 Ketamine Effects on Endocannabinoid System Neurotransmission

It has been suggested that the endocannabinoid system (ES) could also be one of the Ketamine targets involved in the mediation of its antidepressant effects. This hypothesis is supported by the finding that Naltrexone, a non-selective opioid receptor antagonist, blocked the anti-suicidality effects of Ketamine in depressed patients (Williams et al. 2019). A preclinical study in the congenitally learned helpless rat model of depression demonstrated that Naltrexone abolished the antidepressant effects of Ketamine on the cellular hyperactivity observed in the lateral habenula and rat immobility during the FST (Klein et al. 2020). In contrast, another recent, small study showed that pretreatment with Naltrexone did not attenuate the antidepressant effects of Ketamine in patients with MDD (Yoon et al. 2019). Differences between the two studies regarding the route of administration of the compounds and timing of endpoints could explain the observed discrepancies and the potential lack of translational value of rodent data to human studies. In conclusion, further research studies are still warranted to determine the potential role of the ES in the antidepressant effects of Ketamine.

Altogether, main findings from animal models of depression strongly suggest that Ketamine mode of action underlying its rapid antidepressant effects might involve a cascade of events, including the inhibition of NMDARs of GABAergic interneurons followed by increased release (burst) of glutamate and activation of postsynaptic AMPARs. The resulting AMPAR activation leads to enhanced BDNF expression, triggering the mTOR and other downstream intracellular signaling pathways, thus rapidly reversing the atrophy of PFC neurons and restoring PFC disconnectivity caused by chronic stress. However, it is necessary to point out that the effects of Ketamine vary by brain region, circuit, and neuronal type (for instance, pyramidal vs. GABAergic neurons) and differ between Ketamine enantiomers (see Fig. 4.2). A more profound understanding of Ketamine interplay with other

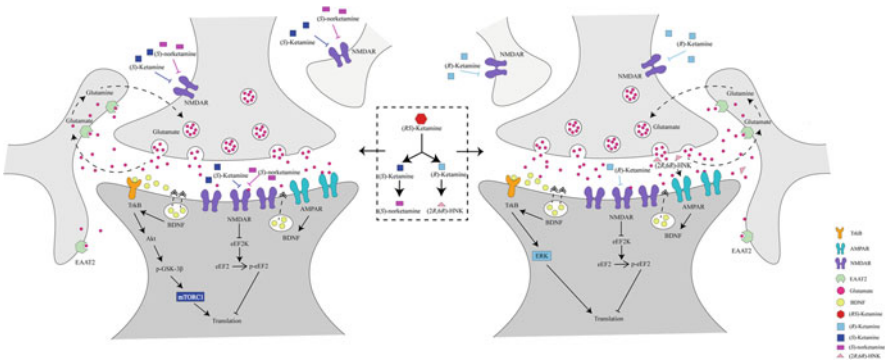


Fig. 4.2 This figure represents the proposed cellular mechanisms of Ketamine enantiomers and metabolites antidepressant actions (from Yang et al. 2019)

neurotransmitter systems might also be necessary for the complete elucidation of its complex mechanism of action.

4.1.2 Ketamine Newly Investigated Mechanisms of Action

Despite extensive preclinical and clinical research efforts in the last few decades, the exact mechanism of Ketamine antidepressant action remains elusive. In this section, we will focus on those recently discovered and deserving further investigation:

(a) Disruption of *circadian rhythm* and sleep pattern is frequently described in patients with MDD.

Studies have reported that patients with MDD have an abnormal expression of circadian clock genes in their ACC (Li et al. 2013). Ketamine enhanced rapid eye movement sleep (Zhuo et al. 2019), and recent work in mice showed that Ketamine induced modification in the expression of genes related to the circadian clock, which could contribute to its antidepressant effects (Orozco-Solis et al. 2017). Those preliminary findings deserve more attention to fully understand the role of circadian rhythm on the antidepressant effects of Ketamine, a question that is being addressed in a recently designed clinical trial (Zhuo et al. 2019).

(b) Research avenues that target the *gut-brain axis.*

Ketamine influence on gut health and its link with mental health and central nervous functions is gaining interest. In particular, Ketamine seems to restore a healthy gut microbiome in mice exposed to chronic stress (Getachew et al. 2018; Qu et al. 2017; Yang et al. 2017).

(c) Another area gaining massive attention from neuroscientists over the last two decades, and experiencing exponential growth of supporting scientific evidence, is related to the role of *inflammation* in MDD etiopathogenesis, which has been well characterized in subsets of depressed patients.

Several studies reported normalization of reactive microglia and increased levels of inflammatory cytokines, including IL-6 in the PFC and HIPP, induced by chronic stress in rodents following Ketamine administration (Tan et al. 2017; Zhang et al. 2020a). Zhang et al. demonstrated that microglia depletion blocked the antidepressant effects of (R)-Ketamine. In the same study, normalization of microglial recombinant transforming growth factor (TGF-β1) signaling in the PFC and the HIPP of chronic social defeat stress susceptible mice by (R)-Ketamine was associated with its rapid and sustained antidepressant effects (Zhang et al. 2020a). Along the same lines, the involvement of heightened IL-6 levels in the development of a depressive phenotype has been consistently supported by the observation that IL-6 deficient mice are resistant to a depressive-like phenotype after exposure to stress (Monje et al. 2011; Ting et al. 2020) and that treatment with IL-6 antibodies improved depressive symptoms in patients with rheumatoid arthritis (Sun et al. 2017). Recent work also

indicated that a subset of patients with treatment-resistant depression displayed additional peripheral inflammation, marked by elevated IL-6 and upregulated chemokines, which were both reduced following Ketamine treatment. Interestingly, among all inflammatory biomarkers analyzed during the study, only fibroblast growth factor two (FGF-2), was found to predict the Ketamine response suggesting that patients with low levels of FGF-2 may be more likely to respond to Ketamine therapy. On the other hand, it is worth mentioning that the levels of inflammatory biomarkers returned to baseline by 24 h after Ketamine infusion and that their decrease did not correlate with clinical response. (Kiraly et al. 2017).

Other authors suggested that Ketamine effects are also mediated through priming of monocytes in an NMDAR- and mTOR signaling-dependent manner, thus initiating an anti-inflammatory cascade of events (Nowak et al. 2019). Similarly, in a cell model of MDD, where PC12 cells (type of catecholamine cells that synthesize, store, and release norepinephrine and dopamine) were treated with corticosterone, Ketamine reduced inflammation, increased viability, decreased the apoptosis of the cells, and increased levels of Krebs cycle enzymes (Zhang et al. 2020b). Finally, in a study by Park et al. Ketamine reduced levels of soluble tumor necrosis factor receptor 1 (sTNFR1), an inflammatory marker found to be correlated with the severity of depression. However, as in the study mentioned before, these changes did not correlate with the extent of patients' clinical improvement, so definitive conclusions about the relationship between Ketamine antidepressant effects and proinflammatory cytokines could not be drawn (Park et al. 2017).

(d) Chronic stress is leading to *oxidative damages of cells*.

Oxidative stress is associated with free radicals that contribute to the pathology of several diseases, including MDD. Some reports indicate that MDD can increase reactive oxygen species (ROS) generation, leading to oxidative damage in limbic brain regions. In a study that used the chronic unpredictable mild stress (CUMS) model of depression, Ketamine was found to reverse the stress-induced increase in malondialdehyde (MDA), a widely used marker of oxidative lipid injury caused by environmental stress, in the PFC, but not in the amygdala (AMG) and HIPP (Maciel et al. 2018).

(e) *Mitochondrial abnormalities* have been implicated in the pathobiology of mood disorders.

Recent advances in metabolomics have allowed scientists to identify pathways significantly altered by Ketamine without *a priori* hypothesis. For instance, 2 h after an injection of Ketamine in mice, several HIPP metabolites relevant for mitochondria functioning were significantly changed, showing that a single injection of Ketamine might impact the main energy metabolism pathways (Weckmann et al. 2014).

4.1.3 Ketamine Effects on Functional and Structural Connectivity-Related Biomarkers in Chronic Stress-Induced Animal Models of Depression and MDD Patients

4.1.3.1 Ketamine Effects on Functional Connectivity-Related Biomarkers

Aside from gross structural changes, the main clinical biomarkers of depression relate more specifically to prefrontal excitability, connectivity, synaptic strength, and changes in neurotrophic factors like BDNF. That is why the latest studies conducted to identify the neuronal pathways modified by Ketamine started incorporating novel imaging techniques such as functional magnetic resonance (fMRI) in their protocols. For instance, Maltbie et al. demonstrated that Ketamine exerted robust and consistent effects at the whole-brain level, primarily increasing functional connectivity in executive control neuronal circuits (Maltbie et al. 2017). Another fMRI study reported a surge in the global prefrontal connectivity during Ketamine infusion, with sustained effects for at least 24 h post-infusion in MDD patients (Abdallah et al. 2018b). Moreover, Grimm et al. found increased PFC-HIPP coupling in healthy humans and rats (Grimm et al. 2015).

In the same way, functional connectivity between the default-mode network (DMN) and ACC, insula, posterior cingulate cortex (PCC), NAc, and the PFC was enhanced by Ketamine in healthy subjects (Fleming et al. 2019). Magnetoencephalography and electroencephalography studies in human subjects suggested that synaptic potentiation via enhanced cortical excitability and increased neuronal plasticity contribute to Ketamine antidepressant effects (Cornwell et al. 2012; Duncan et al. 2013). Along the same lines, a recent 7T-fMRI study showed that Ketamine might indirectly affect the functional connectivity of neuronal networks through BDNF. Woelfer et al. demonstrated that increased BDNF blood levels correlated with decreased functional connectivity between DMN regions usually increased in MDD patients and associated with a ruminative cognitive style. As suggested by the authors, this Ketamine-induced change in functional connectivity may reflect enhanced synaptic plasticity, mediated by increased BDNF levels (Woelfer et al. 2020).

Studies in rodent models of depression have mainly supported findings from human studies mentioned above. For instance, subanesthetic doses of Ketamine increased activation of the mPFC, HIPP, and cortico-limbic regions, as shown in pharmacological magnetic resonance imaging (phMRI) in awake rats (Chin et al. 2011). Using in vivo imaging in iGluSnFR (genetically encoded fluorescent glutamate indicators that enable visualization of neurotransmitter release and diffusion in intact tissue)-expressing mice, McGirr et al. showed that chronic social defeat stress-induced glutamate functional hyperconnectivity was reduced 24 h after Ketamine treatment (McGirr et al. 2017).

Animal studies also demonstrated significant differences in Ketamine antidepressant effects between a single and multiple-dose administration. For instance, Gass et al. showed that Ketamine induced rapid topological modifications in cognitive, sensory, emotional, and reward-related circuitry of rats selectively bred to display a depressed, negative cognitive state (so-called "*negative cognitive (NC) model*", which is one of the most robust and well-validated animal models of treatment-resistant depression), so that 48 h post-injection, the "*depressed*" rats still experienced normalization in functional connectivity of habenula, midline thalamus, and HIPP (Gass et al. 2019). In contrast, in a multiple-dose study using the above paradigm, the same author reported that Ketamine reduced prefrontal connectivity in the NC rat strain, a finding typically observed in patients with MDD (Gass et al. 2020).

4.1.3.2 Ketamine Effects on Structural Connectivity-Related Biomarkers

Depression has often been associated with gross structural changes in the brain, including neuronal atrophy and loss of synaptic connections. Thanks to access to sophisticated imaging techniques, the effect of Ketamine on those changes in defined brain regions can be systematically analyzed in human subjects. Several areas involved in the regulation of emotions have been investigated after treatment with Ketamine. For instance, volumetric changes of the left AMG and the right HIPP after six infusions of Ketamine in patients diagnosed with MDD (Zhou et al. 2020). Moreover, Abdallah et al. showed that small HIPP size was associated with the rapid antidepressant effects of Ketamine (Abdallah et al. 2015a). In the same way, a single infusion of Ketamine normalized enlarged NAc volume in MDD patients (Abdallah et al. 2017).

To assess Ketamine effects on synaptic integrity and plasticity, neuroscientists are predominantly using animal models, as they enable them to perform a finer scale of analysis compared to human in vivo studies. For instance, rodent stress models of depression (e.g., using chronic mild stress or social defeat stress model) display overall synaptic depression in the PFC and HIPP as seen in depressed patients (Abe et al. 2019; Abdallah et al. 2015b). Furthermore, most findings reported that Ketamine leads to changes in dendritic spines in the PFC and HIPP. For example, Ketamine induced a long-lasting (up to 2 weeks) increase in spine density, mainly due to elevated spine formation rate rather than a reduced rate of spine elimination (Phoumthipphavong et al. 2016), and inhibited stress-induced spine loss in rodent models of depression (Ng et al. 2018; Tornese et al. 2019; Ardalan et al. 2017; Zhang et al. 2019). Importantly, changes in spines induced by Ketamine seem to be essential for its sustained antidepressant effects (lasting 2–7 days post-treatment in mice exposed to chronic stress). Indeed, the rapid behavioral effects of Ketamine on stressed mice precede changes in spine formation (Moda-Sava et al. 2019).

It is worth mentioning that Ketamine-induced synaptogenesis is also associated with dynamic vascular plasticity changes such as microvascular elongation. These changes in vascularization might directly support increased neuronal metabolism

induced by neuroplasticity by more intensively transporting oxygen and nutrients to newly generated neurons and synapses (Ardalan et al. 2017).

Multiple studies demonstrated that these neuronal and vascular structural changes are accompanied by functional changes, including increased long-term potentiation (LTP) (Aleksandrova et al. 2020; Sattar et al. 2018), decreased hyperpolarization-activated cyclic nucleotide-gated channel expression in dorsal CA1 neurons of the HIPP (Kim and Johnston 2020), and increased spike frequency of individual neurons of the ventromedial PFC (Abe et al. 2019).

A crucial avenue related to Ketamine research pursued by neuroscientists concerns the intracellular mechanisms by which Ketamine restores synaptic integrity in the rodent brain following exposure to chronic stress. The two primary signaling pathways underlying the rapid antidepressant actions of Ketamine that have been consistently investigated are the BDNF/TrkB- and mTOR-dependent pathways. Both are directly implicated in synaptic dysfunctions observed in depression and chronic stress rodent models, whereby stress-related synaptic deficits are precipitated by a reduction in BDNF and by inhibition of mTORC1 (Abdallah et al. 2015b; Chandran et al. 2013; Colucci-D'amato et al. 2020).

Ketamine induces the rapid release of BDNF, which binds to the TrkB receptor, mediating its neurotrophic and neuroplastic effects (Liu et al. 2016; Duman et al. 2021; Zhang et al. 2018a). This mechanism of action was further confirmed in BDNF and TrKB knockout mice that failed to display antidepressive-like behaviors in response to Ketamine injection (Autry et al. 2011) and also in a rat model of chronic unpredictable mild stress (CUMS) where the use of ANA-12, a TrkB antagonist, blocked the antidepressant effects of Ketamine (Sun et al. 2016). Anderzhanova et al. also demonstrated that the presence of the stress-responsive glucocorticoid receptor co-chaperone FK506 binding protein 51 (FKBP51) in the PFC is necessary for (S)-Ketamine to induce the secretion of mature BDNF and for exhibiting its acute antidepressant effects (Anderzhanova et al. 2020). Moreover, in FKBP51 knockout mice, acute antidepressant treatment failed to show an effect, further corroborating the previous reports (Gassen et al. 2014). These preclinical findings are supported by human studies showing that levels of FKBP51 (Gassen et al. 2014) can predict treatment response in depressed patients. Other researchers showed that the rapid antidepressant effects of Ketamine, driven by increased BDNF translation, are dependent on inhibition of eukaryotic elongation factor 2 kinase (eEF2K), which is sufficient to elicit an antidepressant response in mice (Autry et al. 2011). The sustained effects of Ketamine are also mediated by BDNF/TrkB signaling. In the study by Ma et al. Ketamine accelerated the maturation of neural progenitor cells in the HIPP, a mechanism that requires TrKB activation (Ma et al. 2017).

In the same way, Ketamine recovered HIPP levels of the protein p11 (a member of the S100 EF-hand protein family that generally modulates cellular target proteins in response to intracellular Ca^{2+} signals) in rats exposed to CUMS. This finding was further supported by Sun et al. who demonstrated that the knockdown of p11 in the rat HIPP led to depressive-like behaviors that were not improved by Ketamine (Sun et al. 2016). Besides BDNF, other growth factors have been implicated in the

antidepressant effects of Ketamine, including vascular endothelial growth factor (VEGF), insulin-like growth factor 2 (IGF2), and transforming growth factor (TGF)-β (Zhang et al. 2020a; Duman et al. 2021; Deyama et al. 2019; Grieco et al. 2017; Grossert et al. 2019; Jiang et al. 2019).

All data mentioned before strongly support the hypothesis that the neurotrophic response associated with Ketamine treatment plays a significant role in its rapid and sustained antidepressant effects (Deyama and Duman 2020).

4.1.4 Other Downstream Pathways Related to Ketamine Antidepressant Effects

mTORC1—In 2010, Duman's group demonstrated that Ketamine rapidly activated the mammalian target of rapamycin (mTOR) in the PFC, increasing the numbers of synaptic proteins and the number and function of new spines and that inhibiting mTOR (using, for instance, Rapamycin) blocked synaptogenesis and the antidepressant properties of Ketamine (Li et al. 2010; Holubova et al. 2016). These preclinical findings highlighted for the first time the involvement of the mTOR signaling pathway in the antidepressant effects of Ketamine. Moreover, Ketamine-induced inhibition of NMDARs initiated a cascade of molecular events, including decreased nitric oxide synthesis, ultimately leading to enhanced mTOR signaling (Harraz and Snyder 2017). It is worth mentioning that the pathway downstream of mTOR involves inhibition of eukaryotic initiation factor 4E-binding protein (4E-BP), which is responsible for inducing HIPP synaptic plasticity associated with its antidepressant effects observed at the behavioral level in mouse models (Aguilar-Valles et al. 2021). Interestingly the cellular mechanisms of action of two Ketamine enantiomers seem to be different. While the antidepressant effects of (S)-Ketamine are dependent on the mTOR signaling pathway, those of (R)-Ketamine rely on the ERK pathway (Yang et al. 2018b) and its long-lasting effect on sustained activation of BDNF/TrkB cascade (Hashimoto 2020).

In contrast, a recent study by Abdallah et al. (2020) in depressed patients did not replicate preclinical findings as a single dose of Rapamycin pretreatment failed to block antidepressant action of Ketamine, apparently extending Ketamine antidepressant effectiveness, in some patients. The authors hypothesized that the synergistic effects of Rapamycin noted in humans might occur due to its specific anti-inflammatory and autophagic effects, which are protecting and promoting neuroplastic changes induced by Ketamine. Preclinical studies additionally emphasized that the effects of Rapamycin (and therefore of mTORC1 signaling) on emotional behaviors might be task-dependent (Holubova et al. 2016). Altogether, these clinical and preclinical studies highlight the need for more intensive investigation of the exact role of mTOR in depression pathology.

The complete cellular and molecular mechanisms underlying the antidepressant effects of Ketamine are still under investigation, and other potential contributors

have been recently identified. For instance, glycogen synthase kinase-3 (GSK3) seems to play an integral role in the rapid antidepressant effect of Ketamine (Costi et al. 2015). Ketamine inhibited GSK3 in a mouse model of learned helplessness, while high doses of Lithium, a GSK3 inhibitor, produced antidepressant effects (Beurel et al. 2011). Similarly, others reported that pretreatment with Lithium of mice exposed to chronic stress potentiated the antidepressant, synaptogenic, and electrophysiological effects of Ketamine (Liu et al. 2013) and decreased the oxidative stress associated with Ketamine use (Chiu et al. 2015). Iadarola et al. demonstrated that the inhibition of GSK3 by Ketamine is mediated by serine phosphorylation in an Ak thymoma/protein kinase B-dependent pathway (Iadarola et al. 2015).

Other potential mechanisms of Ketamine action include those that might be similar to the effects of reelin (a large secreted extracellular matrix glycoprotein that modulates synaptic plasticity by enhancing the induction and maintenance of LTP), as Ketamine was able to rescue the deficits of reelin in a rat model of chronic exposure to corticosterone (Johnston et al. 2020). Other potential downstream pathways include the JAK2/STAT3 signaling pathway (Patton et al. 2017) and the Protein kinase Mζ (Yan et al. 2018).

In conclusion, evidence from humans and rodent models of depression supports the idea that Ketamine antidepressant effects derive from promoting synaptic plasticity and synaptogenesis in brain regions that regulate emotions. Although the complete mechanisms by which Ketamine exerts those effects are still under investigation, they likely involve suppression of resting NMDAR activity leading to inhibition of eEF2 kinase and subsequent dephosphorylation of eEF2 followed by augmentation of BDNF synthesis, activating the mTORC1 pathway to promote synaptogenesis (Henderson 2016).

4.1.5 Ketamine Administration to Prevent Stress-Induced Depressive Symptoms

Recent work in rodents has suggested that using Ketamine preemptively can reduce stress-induced depressive symptoms. Administration of Ketamine 1 week before exposure to stress prevented the occurrence of behavioral deficits relevant to a depressive phenotype in various models of chronic stress in rats and mice. This includes absence of despair or anhedonic behaviors after chronic social defeat (Brachman et al. 2016; Mastrodonato et al. 2018), learned helplessness (Brachman et al. 2016), chronic corticosterone treatment (Brachman et al. 2016; Chen et al. 2020), chronic unpredictable stress (Krzystyniak et al. 2019; Okine et al. 2020), contextual fear conditioning (CFC) (Chen et al. 2020; McGowan et al. 2017, 2018), or inescapable tail shock (Amat et al. 2016; Dolzani et al. 2018). The effects of prophylactic Ketamine on promoting stress resilience could be mediated by changes in synaptic plasticity and neuronal activity in brain regions highly involved in

emotion regulation. For instance, Ketamine increased the formation of dendritic spines in the PFC and CA3 area of the HIPP (Krzystyniak et al. 2019) and increased ΔFosB expression, a neuronal marker associated with resilience to stress, in the ventral dentate gyrus and ventral CA3 region of the HIPP in mice exposed to chronic social defeat stress (Mastrodonato et al. 2018). Other researchers demonstrated that Ketamine might modulate stress resilience through changes in 5-HT transmission (Amat et al. 2016; Dolzani et al. 2018). This idea is supported by Chen et al. (2020), who reported that 5-HT4 receptor agonists exerted a similar effect on resilience as Ketamine when given prophylactically. Other components of glutamatergic signaling exhibited similar stress resilience-inducing properties. For instance, reducing mGlu2R activity prior to inescapable shock stress in mice prevented the development of escape deficits (Highland et al. 2019).

It is worth mentioning that the prophylactic effects of Ketamine seem to depend on several parameters such as the timing of injection vs. the stress exposure, the dose, and sex. McGowan reported that preventive treatment with Ketamine administered 1 month or 1 h before the foot-shock paradigm had no effect on conditioned contextual freezing behaviors in mice, suggesting a time window of 1 week as optimal prophylaxis time (McGowan et al. 2017). Moreover, prophylactic Ketamine consistently protected males from stress-induced emotional dysregulations. In contrast, inconsistent findings in females (Okine et al. 2020; Dolzani et al. 2018) deserve further investigations. Most importantly, the beneficial effects of Ketamine are noted only in stressed animals, as several research groups reported that control rodents do not exhibit the same changes after Ketamine treatment. This finding might suggest that Ketamine is primarily interacting with stress-induced changes in the brain to promote resilience.

4.1.6 Ketamine Use for Other Stress-Related Disorders

Based on the positive results shown by Ketamine for the treatment of MDD, its potential therapeutic effects in other psychiatric conditions have been continuously investigated.

Several reports in rodents indicate that subanesthetic doses of Ketamine can help in treating certain aspects of morphine and amphetamine addiction. For instance, mice treated with Ketamine failed to develop a conditioned place preference for morphine (McKendrick et al. 2020). Along the same lines, amphetamine-induced BDNF expression changes in the NAc and HIPP were rescued by Ketamine in rats (Fuller et al. 2015). However, due to the small number of studies, it remains challenging to draw any definite conclusions on using Ketamine for substance use disorders.

On the contrary, a significant amount of research has been conducted to evaluate the potential benefits of Ketamine in treating fear-related behaviors and post-traumatic stress disorder (PTSD). Studies in rodent models of PTSD and fear indicate that Ketamine alleviated fear generalization and promoted fear extinction

(Asim et al. 2020; Girgenti et al. 2017; Wei et al. 2020b). According to the authors, those effects were probably mediated by Ketamine effects on synaptic plasticity and functional connectivity, likely through a BDNF-dependent pathway within the HIPP, basolateral amygdala (BLA), and PFC (Asim et al. 2020; Krystal et al. 2017; Pradhan et al. 2016; Zhang et al. 2015). It is worth mentioning that significant discrepancies between preclinical studies have been observed, which could be explained mainly by individual variability in susceptibility to stress-related disorders, route, dose, and timing of Ketamine administration (Choi et al. 2020). While data from clinical trials in this field are still scarce, they are however promising. For instance, a small randomized clinical trial by Feder et al. showed a significant reduction in PTSD symptom severity in patients one day after the single-dose intravenous Ketamine infusion (Feder et al. 2014).

In conclusion, the overall evidence points toward a solid therapeutic potential of Ketamine in the context of PTSD, but also for other anxiety disorders, including obsessive-compulsive, generalized anxiety, and social anxiety disorder (Taylor et al. 2018).

4.1.7 Comparative Analysis of Ketamine vs. Ketamine-Like Drugs and Other Glutamatergic Compounds

Before Ketamine introduction, depressed patients were most often prescribed monoamine-based antidepressants. In a comparative study in mice, using the chronic social defeat stress model, Bagot et al. demonstrated that both Ketamine and the monoaminergic antidepressant Imipramine mainly exerted their antidepressant effects by regulating the expression of genes modulating resilience mechanisms in the PFC (Bagot et al. 2017). However, significant differences in target engagement between these two drugs were also noted, with Ketamine regulating gene expression within the NAc and Imipramine acting primarily on the HIPP.

The benefits of a recent FDA and EMA regulatory approval of Ketamine for indication of treatment-resistant MDD (TRD) are twofold. Firstly, a glimpse of hope has been given to all TRD patients that there is an antidepressant to help them overcome cumbersome symptoms and regain a positive mood quickly, and at the same time assure long-term mental well-being and quality of life. The second benefit pertains to the mental health professionals as their antidepressant armamentarium was enriched with an innovative, highly effective treatment modality with rapid onset of action, which could reverse brain changes induced by depression, including those in the difficult-to-treat subgroup of patients. Although promoted as the most significant breakthrough in depression treatment in half a century, Ketamine use in humans is severely limited, mainly due to safety issues related to its sedative, dissociative, and psychotomimetic properties, including a high risk for abuse and dependence, and possible neurotoxicity (Shin and Kim 2020). Moreover, others have reported that Ketamine can exert opposite effects when given to non-stressed

healthy individuals mimicking a stress-like response by increasing the stress hormone cortisol levels, affecting essential physiological and psychological functions (Khalili-Mahani et al. 2015a, b). Chronic use and abuse of Ketamine in Ketamine-dependent individuals has also been associated with white matter abnormalities in frontal and temporoparietal cortices (Liao et al. 2010) and neuronal projections between the caudate nucleus and PFC (Edward Roberts et al. 2014). Indeed these anatomical changes were associated with spatial memory impairments and altered HIPP activation in the same population (Morgan et al. 2014). These findings are supported by preclinical data showing that prolonged exposure to Ketamine deteriorates spatial memory in rats. However, these results were observed only in control, non-stressed rats, while stressed rats benefited from chronic Ketamine use (Trofimiuk et al. 2019; Yang et al. 2018c). All the limitations mentioned above led scientists and clinicians to investigate and develop alternative, safer treatment modalities that would mimic Ketamine fast-acting and sustained antidepressant properties.

Armed with the knowledge that Ketamine mainly exerts its antidepressant effects through modulation of glutamatergic neurotransmission, the researchers initially started developing similar glutamate-based compounds. The first clinical trials with Ketamine already noted differences between (R)-Ketamine and (S)-Ketamine isomers. For instance, although both enantiomers share a downstream pathway through the activation of mTORC1 signaling, (S)-Ketamine has a higher affinity for the NMDAR and is believed to be responsible for the abuse-related effects of Ketamine. While (S)-Ketamine activates the AMPAR and is metabolized to (S)-norKetamine (Yang et al. 2019), (R)-Ketamine is more potent, exerting long-lasting antidepressant effects, and shows a better side effect profile, at least when used at a low dose (e.g., 20 mg/kg in mice) (Chang et al. 2019; Yang et al. 2015; Zanos and Gould 2018; Zanos et al. 2019a). Furthermore, it also activates AMPARs and promotes MEK-ERK and BDNF-TrkB signaling (Yang et al. 2019). (R)-Ketamine is metabolized to (2R,6R)-hydroxynorketamine (HNK) in the liver, and subsequently, as (2R,6R)-HNK crosses the blood-brain barrier (Zhang et al. 2018b). It is currently thought that (R)-Ketamine main antidepressant properties are mediated by the same metabolite (Elmer et al. 2020; Hillhouse et al. 2019; Hashimoto 2019), although this claim was recently questioned, as Zhang et al. (2018b) demonstrated in a mouse model of CSDS that (2R,6R)-HNK is not necessary for (R)-Ketamine antidepressant action. Other research groups continued to investigate the mechanisms of action underlying (2R,6R)-HNK antidepressant properties and reported that (2R,6R)-HNK inhibited synaptic NMDARs, triggering a cascade of intracellular events that could explain the long-lasting antidepressant effects seen with Ketamine (Zanos et al. 2016; Suzuki et al. 2017). Along the same lines, a recent study by Zanos et al. also indicated that the mechanisms underlying the antidepressant effects of (2R,6R)-HNK converge with mGlu2 receptor signaling (Zanos et al. 2019b), while others reported that some of its downstream intracellular effects involve BDNF (in the PFC) (Fukumoto et al. 2019) and glucocorticoid receptor signaling (Herzog et al. 2020). Different pathways related to (2R,6R)-HNK implicate signal transducer and activator of transcription 3 (STAT3), a protein involved in immune response,

and its interaction with eukaryotic elongation factor 2 (EEF2), a protein responsible for synaptic plasticity and memory consolidation, resulting in the augmentation of BDNF expression and promotion of postsynaptic density protein 95 (PSD95) and synapsin I (SYN1) synthesis (Ho et al. 2019). Interestingly, some researchers reported that antidepressant-relevant concentrations of (2R,6R)-HNK were insufficient to block NMDARs, suggesting that direct inhibition of NMDARs did not contribute to its antidepressant effects (Lumsden et al. 2019). Indeed, Riggs et al. demonstrated that (2R,6R)-HNK led to presynaptic potentiation in the HIPP of rats that did not require NMDAR activation and was not impeded by NMDAR inhibitors (Riggs et al. 2020). Altogether, currently available data indicate that therapeutic approaches based on (2R,6R)-HNK might be as efficient as those with Ketamine in terms of rapid and sustained antidepressant effects but would be associated with fewer adverse events (Chaki 2017).

Other NMDAR antagonists have been evaluated for their effects in MDD and compared to Ketamine. For instance, the low trapping non-selective NMDA channel blocker Lanicemine (AZD6765) was shown to exert rapid antidepressant effects in patients with TRD. Unfortunately, these effects were short-lived (Zarate et al. 2013). Lanicemine also increased prefrontal global connectivity 24 h post-treatment in depressed patients; noteworthy, the extent of the increase was not as significant as the one observed after Ketamine infusion (Abdallah et al. 2018b). Similar to Ketamine, psychotomimetic effects were also observed following Lanicemine administration (Sanacora et al. 2014), and a recent double-blind placebo-controlled clinical trial using 15 intravenous infusions of 50 mg or 100 mg of Lanicemine over 12 weeks in patients with MDD and a history of inadequate response to antidepressants by Sanacora et al. failed to show the statistically significant difference when compared to placebo (Sanacora et al. 2017).

Another NMDAR antagonist, MK-801, induced antidepressant effects in mice, but its effects did not persist beyond 3 h post-treatment (Autry et al. 2011). Moreover, administration of CP-101,606, an NR2B-selective NMDAR antagonist, was associated with psychotomimetic side effects similar to those observed with Ketamine (Preskorn et al. 2008).

Another promising glutamatergic compound tested in human clinical trials is Rapastinel (also known as GLYX-13), a partial agonist to the glycine site of the NMDAR. Initial clinical trials reported that GLYX-13 simultaneously enhanced the magnitude of LTP of synaptic transmission while reducing long-term depression (LTD) in Schaffer collateral-CA1 synapses in rat HIPP slices, suggesting its unique pro-cognitive properties for the first time in 2008 (Lei et al. 2008). Subsequent animal studies showed its long-lasting antidepressant effects in rats, associated with dendritic spine morphology and long-term synaptic plasticity changes (Burgdorf et al. 2015; Donello et al. 2018; Moskal et al. 2014). These findings were partially replicated in a mouse model of social defeat stress, showing rapid antidepressant effects of Rapastinel similar to R-Ketamine. On the other hand, (R)-Ketamine produced longer-lasting antidepressant effects than Rapastinel in a comparative study (Yang et al. 2016).

A proof of concept study by Preskorn et al. in patients with MDD nonresponsive to a previous antidepressant, showed Rapastinel decreasing depressive symptoms within hours of treatment, with effects sustained for up to 7 days (Preskorn et al. 2015). It was suggested that antidepressant properties of GLYX-13 are mediated by activation of voltage-dependent calcium channels, the release of BDNF and stimulation of TrkB in the PFC and HIPP (Yang et al. 2016; Kato et al. 2018), and activation of the mTORC1 pathway in the PFC (Liu et al. 2017). The scientific explanation about the lack of psychotomimetic side effects associated with Rapastinel use when compared to Ketamine, according to Li et al. may be related to their differences pertaining to Rapastinel's lack of affinity for 5-HT2A receptors (Liu et al. 2017). However, recent studies with psychedelics suggested a completely different role for the 5-HT2A receptors in mediating neuroplasticity effects of hallucinogenic drugs (Ly et al. 2018).

Finally, another glutamatergic compound with antidepressant effects is nitrous oxide (N_2O), a non-competitive NMDAR antagonist, which psychotropic properties were described for the first time during the last years of the eighteenth century by Humphry Davy. N_2O proved to be clinically effective in lifting reactive but not endogenous depression in a psychiatric study in 1928 by Dr. Julius Zador, a Hungarian psychiatrist working at the Greifswald University in Germany. More recently, in a proof-of-concept trial published in 2014, Nagele et al. reported that N_2O reduced depressive symptoms in treatment-resistant depressed patients in a double-blind, placebo-controlled, cross-over study. The N_2O showed rapid antidepressant effects, sustained for at least 24 h up to 1 week, with 20% of patients classified as treatment responders and 15% who achieved remission (Nagele et al. 2015). Aside from targeting NMDARs, the antidepressant mechanisms of action of N_2O could also involve inhibition of low voltage-activated calcium channels and two-pore-domain potassium channel (TREK-1) (Kalmoe et al. 2020).

However, the exact impact of the N_2O on brain circuits remains elusive. For instance, in the HIPP of rats, N_2O disinhibited population spike firing (Nagashima et al. 2005), an effect similar to the one observed with Ketamine in the cortex (Zorumski et al. 2015). In human brains, electro-encephalographic recordings revealed that the N_2O dampened functional connectivity in superficial parietal layers (Kuhlmann et al. 2013). Altogether, there is an overall lack of consistent information on the exact effects of the N_2O on the functional connectivity of neuronal networks in the depressed brain (Zorumski et al. 2015). While relatively safe, chronic use of the N_2O can lead to neurological damages, potentially limiting its use as an established treatment for depression.

Other treatment modalities increasing glutamatergic transmission by facilitating its reuptake by glial cells have also been assessed for their potential antidepressant effects. For instance, Riluzole, an FDA-approved drug for treating amyotrophic lateral sclerosis, is one of these compounds. Indeed, Riluzole reversed the depressive-like phenotype (helplessness and anhedonia) and glial dysfunction in rats exposed to CUMS (Banasr et al. 2010), similarly to Ketamine, by upregulating the expression of Na+-dependent excitatory amino acid transporters (EAATs) in chronically stressed rats (Zhu et al. 2017). These studies further highlight the

importance of glial cells in the neurobiology of MDD, underlining their therapeutic potential for successfully managing mood disorders.

Other glutamatergic compounds targeting a different set of glutamate receptors have also been tested to treat stress-related disorders such as depression. For instance, mGluR2/3 antagonists showed Ketamine-like rapid and sustained antidepressant properties in rodent models (Dwyer et al. 2013). These effects are thought to be mediated by synaptogenesis in the PFC and HIPP and/or modulation of the serotonergic system (Chaki and Fukumoto 2019; Dong et al. 2017). Moreover, combining antagonists of the mGluR2/3 receptors with (2R,6R)-HNK led to synergistic effects characterized by anti-depressive-like behaviors in mice and increased cortical EEG gamma-oscillations (or "*rhythms,*" suggested as one of the potential biomarkers of MDD) (Zanos et al. 2019b). Other pharmacological approaches looked further into targeting the GluN2B subunit of the NMDARs directly since it has been shown that this subunit is necessary for mediating the antidepressant effects of Ketamine. For instance, Ro 25-6981, a selective negative allosteric modulator of GluN2B, showed rapid and sustained antidepressant properties that could be partially mediated by its inhibitory effect on monoamine reuptake (Talbot et al. 2016). For comparison, a GluN2A receptor antagonist, NVP-AAM077, also led to antidepressant effects in rats, but unfortunately, they did not persist and entirely disappeared 7 days post-treatment (Gordillo-Salas et al. 2018). Other compounds with less specific NMDAR antagonist properties, like Methadone, produced rapid antidepressant effects through the same downstream mTORC1-dependent mechanism in the PFC as Ketamine (Fogaça et al. 2019). Finally, chronic AMPA treatment, including AMPA-Ketamine combination, was associated with antidepressant effects and increased markers of HIPP neurogenesis and synaptogenesis in the Wistar Kyoto rat (Akinfiresoye and Tizabi 2013), an animal model extensively used to assess the efficacy of antidepressants.

Targeting the mTOR signaling cascade with other compounds also efficiently rescued depressive-like phenotype. For instance, Yueju (a Chinese medicinal herb) improved anhedonic behaviors and increased body weight in chronically stressed mice (Tang et al. 2015), while Scopolamine, a non-selective muscarinic acetylcholine receptor (mAchR) antagonist, increased mTORC1 signaling in the PFC of mice and reduced immobility time in the FST (Voleti et al. 2013). These additional findings further demonstrate the importance of the mTORC1 signaling pathway in inducing rapid and sustained antidepressant effects.

Interestingly, other researchers have suggested that the antidepressant effects of both Ketamine and Scopolamine could also be mediated by small conductance calcium-activated potassium channels, revealing a potential target for developing novel therapeutics for MDD (Bambico et al. 2020).

Finally, neuroscientists have recently started to pursue other research avenues investigating, for instance, the role of the GABAergic system in depression. As such, negative allosteric modulators of the α5-containing $GABA_A$ receptors displayed similar effects as Ketamine in rodent models of chronic stress, primarily replicating its fast-acting properties at the behavioral and synaptic levels (Fischell et al. 2015; Xiong et al. 2018).

4.1.8 Limitations of Current Knowledge on Ketamine Use in MDD

Our current knowledge on the mechanisms of action of Ketamine and its short- and long-term effects is still incomplete and often subject to inconsistencies (Polis et al. 2019). These limitations are due to various factors that will be discussed here.

First and foremost, the vast majority of what we know on Ketamine effects on neuronal circuits and cellular mechanisms involved in emotional regulation is based on preclinical models that rarely recapitulate all aspects of depression (Ramaker and Dulawa 2017). Moreover, rodent models often fail to include sex as a biological variable. This limitation is of particular importance for two reasons: (1) females are more sensitive to stress-induced changes in emotional behaviors. This increased stress vulnerability at the behavioral level noted in animal models parallels nicely with human data showing an increased risk for stress-related disorders in women and is also evidenced by female-specific changes in gene expression in the PFC following stress exposure in mice (Barko et al. 2019); (2) there is a lack of studies reporting sex-specific effects of Ketamine. Furthermore, the increased sensitivity of females to Ketamine deserves to be investigated in depth to fully grasp the importance of sex hormones on Ketamine antidepressant properties, including the associated risk for abuse in the female population (Saland et al. 2017; Wright et al. 2019).

Another significant limitation is that most of the work conducted in rodent models is based on a single, acute dose of Ketamine. In contrast, in the human depressed population, the administration of multiple doses is a necessity. A study conducted in rats in 2017 showed that long-term treatment with Ketamine failed to induce antidepressant effects in chronically stressed rats (Jiang et al. 2017). In contrast, Borsoi et al. showed that mice exposed to daily Ketamine for 14 days displayed a reduction in the immobility time in the FST compared to controls (Borsoi et al. 2021). This inconsistency in findings emphasizes the importance of further investigation related to repeated exposure to Ketamine regimen and the possibility of interference with its sustained antidepressant properties. Another critical factor that might be related to the discrepancy related to short- and long-term effects of Ketamine found in previously mentioned animal studies is that Jiang's study (Jiang et al. 2017) was conducted in stressed rodents, while Borsoi's research (Borsoi et al. 2021) was done in non-stressed control mice. The differences observed in those two preclinical studies are in alignment with human studies as it has been reported that the effects of Ketamine are opposite between depressed patients vs. healthy controls (Nugent et al. 2019). Indeed, an fMRI study further supported this finding showing that Ketamine effects on activation of specific brain regions during emotionally valenced attentional processing in MDD patients vs. healthy controls are diametrically opposite (Reed et al. 2018). In conclusion, the use of control non-stressed animals (and by extension of healthy control human subjects) to elucidate antidepressant mechanisms of action of Ketamine is highly controversial as it does not necessarily reflect what would happen in the depressed brain (Fitzgerald et al. 2019).

Altogether, the discovery of the antidepressant properties of Ketamine gave hope to the millions of individuals affected by depression. The fast-acting and sustained effects make Ketamine a unique and probably the most efficient contemporary approach for TRD. In addition, the study of its mechanisms of action confirmed the importance of glutamatergic transmission, neurotrophic factors, and synaptic plasticity in the neuropathology of depression (Pereira and Hiroaki-Sato 2018). Unfortunately, the severe side effects associated with Ketamine use significantly limit its widespread administration. For instance, although the U.S. Food and Drug Administration approved in 2019 Esketamine nasal spray for TRD, it remains only available through a restricted distribution system under a Risk Evaluation and Mitigation Strategy (REMS) because of its sedative and dissociative effects. However, it needs to be emphasized that an even more significant benefit of Ketamine introduction in the field of human psychopharmacology is that it led to the discovery of new Ketamine-like compounds that are currently being tested in difficult-to-treat depressive patients. Hopefully, these new initiatives from both academia and industry members will pave the way for developing and introducing novel, more sophisticated antidepressant agents expressing similar efficacy levels associated with much more benign safety profiles.

4.2 Future Directions for Glutamate-Based Management of Chronic Stress and Stress-Related Disorders

Chronic stress exerts widespread actions in the central nervous system (CNS), ranging from the regulation of gene transcription, cellular signaling, synaptic structure, and neurotransmission to cognition, emotion, and behavior (de Kloet et al. 2005; McEwen et al. 2015). Maladaptive plasticity in stress-vulnerable subjects, in turn, increases the risk of developing major psychiatric and neurodegenerative diseases. However, while chronic exposure to adverse experiences may direct the stress response toward negative health outcomes, proadaptive interventions can re-shape brain circuits, redirecting illness-related trajectories to those associated with enhanced mental and physical well-being (McEwen and Akil 2020).

This section presents strategies that would increase the understanding of the pathophysiological processes underlying (mal)adaptive stress responses, helping us develop interventions that will mitigate the effects of chronic stress and prevent stress-related disorders.

4.2.1 *Improving the Utility and Translation of Animal Models of Chronic Stress-Induced Disorders*

Studies in laboratory animals are essential to allow mechanistic insights into the association between chronic stress and systemic diseases. Ideally, animal models should mimic the natural course of diseases and induce similar stress responses as seen in humans, but, unfortunately, each model of stress suffers from its own limitations (Czéh et al. 2016; Wang et al. 2017; Planchez et al. 2019).

Different nervous and humoral system regions are activated depending on the type, duration, and intensity of stressors. The personality traits and attitudes towards stress also strongly influence pathophysiological responses to stress. Moreover, some symptoms of psychiatric diseases, such as the feeling of guilt, suicidal thoughts, the inability to cope with financial distress, or the loss of a loved one, are not possible to replicate in animals. Overall, this complexity presents many challenges in creating suitable animal models of stress, making the interpretation of animal experiments largely presumptive.

A main confounding factor is that animals and humans have a unique ability to adapt to chronic stresses, thus diminishing the magnitude of adverse reactions over time. In this context, mimicking closely the naturalistic conditions associated with human living and MDD, the unavoidable and unpredictable nature of the stress regimen plays a crucial role in the preclinical modeling of depression (Willner and Mitchell 2002). However, this increases the complexity of the model (difficulty in performing the experiments, which demand substantial work, space, and time) and the risk of losing reliability if not significantly increasing the number of animals included in the study.

While no single animal model presents the ideal system for assessing depressive behavior, different paradigms have advantages over others for studying specific aspects of the disease. For example, the chronic social defeat model has the advantage of addressing core and common symptoms of depression such as long-term anhedonia and helplessness and other clinical aspects highly comorbid with major depression such as anxiety and social avoidance (Patel et al. 2019). However, social defeat is mainly limited to adult male animals and might be difficult to be translated to all strains. On the other hand, chronic mild stress overcomes the issue related to stress prediction and can be applied to both males and females from several different strains. Still, its major drawbacks are that it is laborious and time-consuming (Willner 2005).

Studying resilience is also essential for understanding what causes stress susceptibility (McEwen 2016). Thus, models that include this aspect can significantly benefit us in understanding the consequences of chronic stress. More comprehensive studies of chronic stress may need to use several strains, multiple testing models, cross-compare genetic and epigenetic expressions and activity, and always collaborate with human data, including both ex vivo and postmortem tissues (Willner 2016).

4.2.2 The Heritable Aspect of Environmental Stress

The epigenome (the term derived from the Greek word "*epi*", which means "*above*" the genome) is critical for establishing and maintaining cell identity. At the same time, epigenetic changes are also involved in regulating numerous cellular functions, including adaptation to external changes and chronic stress response (Torres-Berrío et al. 2019).

The idea that epigenetic inheritance can be affected by environmental factors in the absence of DNA mutagenesis and that the resulting changes can propagate to subsequent generations via the germline has been a matter of considerable debate. However, converging compelling evidence suggests that the long-term molecular and functional consequences of chronic stress exposure, together with the associated behavioral phenotype, can be transferable to offspring via epigenetic mechanisms (Bohacek and Mansuy 2015; Zucchi et al. 2012; Jawaid et al. 2018). Importantly, such effects are not limited to the first generation but may be transmitted to a second or several subsequent generations, thus resulting in transgenerational epigenetic inheritance of maladaptive responses to environmental stress, which could potentially affect the risk of developing stress-related systemic disorders.

In humans, the best evidence for transgenerational inheritance induced by environmental factors relates to the effects of diet and food availability in early life. For example, gestational famine has been associated with a higher risk of obesity, glucose intolerance, and coronary heart disease in adulthood (Lumey et al. 2011). Considering that transgenerational human studies are complex because of the time scales involved and the influence of genetic, social, and cultural factors, animal studies have been undertaken to elucidate the underlying mechanisms. Indeed, many preclinical studies have shown that different early-life environmental stressors may impact health and disease risk in subsequent generations, with mechanisms involving alterations in DNA methylation, histone modifications, and small RNAs (Bohacek and Mansuy 2015; Zucchi et al. 2012; Jawaid et al. 2018). These changes occur despite the extensive epigenetic reprogramming occurring in the mammalian germline to ensure the totipotency of the zygote through both the maternal and paternal lines (Fig. 4.3). Importantly, transgenerational inheritance involves alterations in the germline epigenome, thus ensuring the transmission of effects across generations.

Understanding how the effects exerted by chronic stress in one individual may affect subsequent generations may significantly improve successive generations' health prospects and quality of life.

4.2.3 Biomarkers in Stress-Related Disorders

Ongoing research on animal models of stress and the study of clinical outcomes in chronically stressed individuals promise to improve our understanding of trajectories

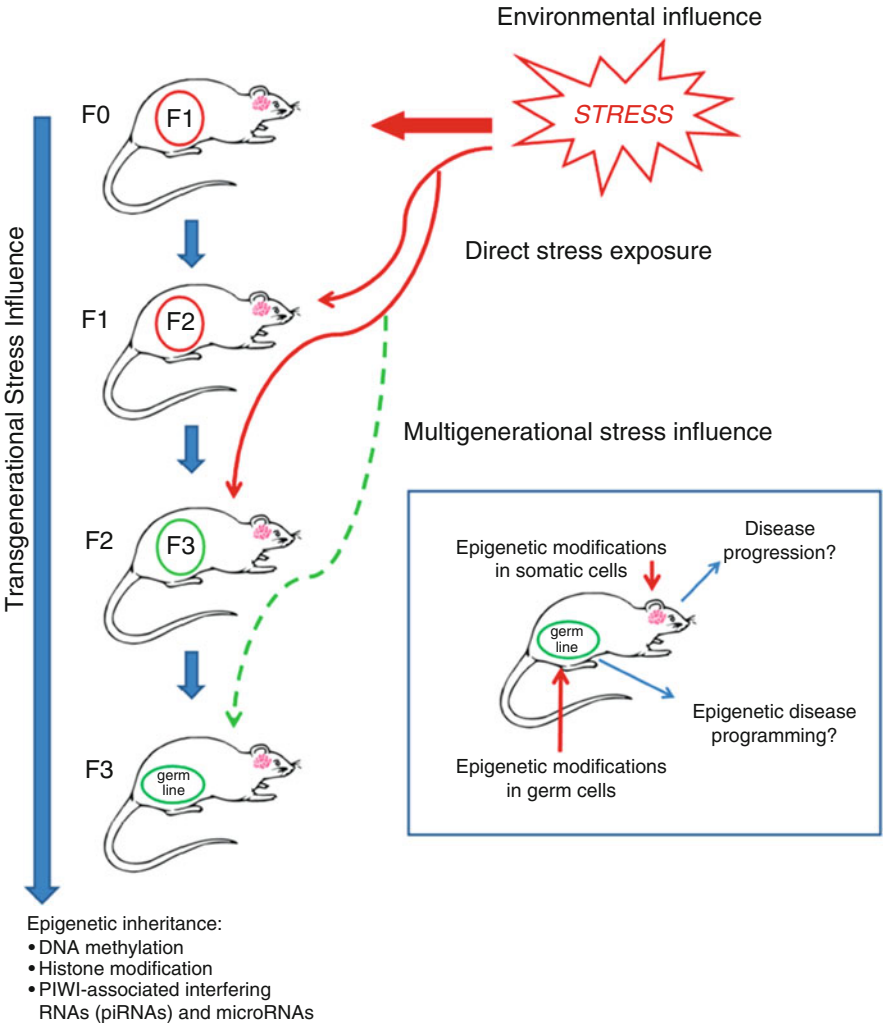


Fig. 4.3 Inheritance of an epigenetic memory caused by stress exposure produces phenotypes in next generations that may influence disease risk. A solid red arrow indicates direct exposure to stress in an animal; thin red arrows indicate direct stress exposure leading to intergenerational stress influence in F1 and F2 generations; a dashed thin green arrow indicates possible transgenerational inheritance of a multigenerational stress phenotype in the F3 generation. Note: it is necessary to determine phenotype transmission to the third generation to prove genuine epigenetic programming (F3) (from Zucchi et al. 2012)

of a stress response. Moreover, identifying molecular and systemic mediators associated with (patho-)physiological stress response can help determine biomarkers (proteins, enzymes, hormones, chemicals, metabolites, genes, or by-products), which may have the potential to be used as effective clinical tools. Indeed, a large body of research has focused on searching for diagnostic biomarkers as indicators

for physiological and pathological processes underlying chronic stress response and predictive biomarkers giving us the information about pharmacological response to therapeutic intervention for stress-related disorders (Dhama et al. 2019).

Classical stress markers comprise endocrine changes, especially in hormones such as cortisol and epinephrine. Other potential biomarkers include thermal stress markers, such as heat shock proteins (HSPs), innate immune markers, such as acute phase proteins (APPs), oxidative stress markers, and chemical secretions within saliva and urine. However, the reaction to environmental stressors differs substantially between individuals, and the stress response cannot be considered universal. Therefore, although various critical molecular signatures have been identified in association with stress-related disorders, identifying the best and most reliable biomarker(s) is crucial because the most promising markers need to be highly correlated with the specific pathophysiological aspects of the clinical condition (Dhama et al. 2019; McEwen 2015). However, due to the complexity of the human body functioning, it would be necessary to consider multiple parameters, possibly covering at the same time potent mediators of the cardiovascular, CNS, hepatic and nephrological disorders, energy dissipation, and anti-oxidative defense, to identify clusters of biomarkers associated with stress vulnerability and onset of different diseases.

The first attempt toward combining such data may involve metabolic modeling studies, which potentially give perspective about multisystem involvement and widespread disturbance in various biomarkers associated with protective and stress pathways (Dhama et al. 2019). From this perspective, the accurate evaluation of stress markers available through non-invasive methods from saliva, urine, tears, and feces will be a critical part of measuring the stress response in humans, thus requiring the inclusion of a wide range of (patho)physiological parameters. Present quantitation methods based on immunological, chromatographic, and mass spectrophotometric assessments, despite some variability, can produce reliable results. However, further scientific and technical advances are necessary to make possible profiling of the markers in each biological sample, providing a broader chance to identify optimal and specific biomarkers of stress-related disorders.

4.2.4 Preventive Nutritional and Behavioral Strategies to Counteract the Effects of Chronic Stress

The relationship between dietary constituents, nutrition, inflammation, and oxidative stress has been well established and plays a significant role in response to chronic stress (Ramos-Lopez et al. 2021). Dietary and nutritional ingredients known to exert anti-inflammatory and antioxidant properties include omega-3 fatty acids, vitamin A, vitamin C, E, and phytochemicals, such as polyphenols and carotenoids. Moreover, plant-based food consumption has been associated with additional health benefits, including anti-inflammatory properties obtained through short-chain fatty

acids, metabolic compounds produced during the fermentation process of carbohydrates (dietary fiber), and vegetal proteins by human intestinal microbiota. Such anti-inflammatory compounds could play an essential role in maintaining overall homeostasis disrupted by inflammation and oxidative stress, both before and during chronic stress exposure (Marx et al. 2017; Sarris 2019).

In contrast, high fat/high-calorie diets and excessive fructose consumption contribute to increasing inflammation (Tan and Norhaizan 2019). Similarly, conditions such as obesity and diabetes are associated with adipose tissue dysfunction and low-grade systemic inflammation (Luppino et al. 2010; Milaneschi et al. 2019). All these factors contribute to the development of cardiovascular disorders and diabetes and increase the risk of infections and susceptibility to chronic stress. Accordingly, many pathways connect exposure to stressful conditions and obesity, including HPA axis activation, changes in feeding behavior, reward processing, microbiome, and cognitive performance.

Physical activity also substantially impacts redox homeostasis, and regular physical exercise reduces the body's stress hormones, such as adrenaline and cortisol (Kandola et al. 2019). While some physical exercise may exert anti-inflammatory effects depending on the type of activity, exercise duration, body composition, gender, and age, excessive physical activity can instead induce inflammation as the exercise itself represents a type of physical stress that challenges homeostasis. Moderate exercise has been consistently shown to bring about many positive physiological changes, resulting in improved mood state, self-esteem, and decreased stress and anxiety levels (Dinas et al. 2011). It seems that the benefits of exercise are the result of numerous physiological and psychological changes, involving increased endorphin levels, body temperature, mitochondrial function and mitochondriogenesis, neurotransmitter production, as well as attenuation of the HPA axis response to stress (Phillips 2017). Moreover, physical exercise increases dopaminergic signaling, particularly in the reward pathway, and promotes glutamatergic neurotransmission-induced neuroplasticity.

Overall, the combination of several lifestyle components, including sleep habits, smoking, alcohol consumption, interacts with the genetic background, shaping an individual's ability to cope with stress. This interplay leads to epigenetic modifications, affecting inflammatory pathways and inducing neuroinflammation (McEwen 2016; Ramos-Lopez et al. 2021). However, due to the heterogeneity of the existing data and the scarcity of studies in humans, additional double-blind placebo-controlled randomized clinical trials in the field of nutriepigenetics are required. Progress in the identification of epigenetically active dietary components and lifestyle factors will strongly contribute to the creation of innovative prophylactic and therapeutic interventions, which will include daily consumption of dietary bioactive compounds, regular physical activity, and other optimal lifestyle behaviors adjusted to the specific population and disease groups.

4.2.5 Nutritional Supplements for the Treatment of Depressive Symptoms

A possible link between specific micronutrient supplementation and its antidepressant effects has also been proposed (Hoepner et al. 2021). Indeed, in line with the evidence described in the previous paragraph, several nutrients, including vitamins, minerals, fatty acids, and essential amino acids, have been implied in the regulation of neurological, hormonal, neurotransmitter, and other signaling pathways related to chronic stress-induced depressive symptoms. Among those, although with mixed results, most of the studies focused on folate, homocysteine, S-adenosylmethionine (SAMe), L-acetylcarnitine, alpha-lipoic acid, N-acetylcysteine, L-tryptophan, zinc, magnesium, vitamin D, omega-3 fatty acids, coenzyme Q10, and inositol.

Some reports also suggested that augmenting conventional antidepressants with "*medical foods*" may be a viable option for individuals with depression who have tried and failed multiple antidepressant regimens. L-acetylcarnitine and L-methylfolate have obtained the most robust body of evidence as putative antidepressant-augmenting agents (Chiechio et al. 2018; Martone 2018). Importantly, preclinical observations demonstrated that antidepressant properties of L-acetylcarnitine involve neuroplastic effects, which are in turn dependent on changes in neurotransmitter regulation and metabotropic glutamate receptor upregulation, further confirming the importance of glutamatergic neurotransmission in the induction of antidepressant effects and neuroplasticity (Nasca et al. 2017). In addition, preclinical evidence showed that other micronutrients, including L-theanine, curcumin, taurine, zinc, magnesium, and selenium, may exert antidepressant/anti-stress effects through mechanisms involving the modulation of glutamatergic transmission.

Although the molecular mechanisms linking nutritional supplementation with changes in central glutamatergic function remain largely unknown, anti-inflammatory and metabolic actions are likely to be involved. Indeed, both inflammation and interference with glutamate metabolism may impact glutamate transmission through processes involving glutamate excitotoxicity, oxidative stress, and apoptosis (Miller et al. 2009).

4.2.6 Conclusion: Dealing with Stress

The stress response involves several systems, ranging from endocrine and immunological functions to cognitive, emotional, and behavioral responses. Thus, the consequence of chronic stress has a relevant impact at several levels, including physical well-being, psychological and sociological aspects. However, combined preclinical and clinical evidence shows that a dysregulated stress response can be redirected or re-tuned, not only through medical approaches but also through psychosocial strategies, as well as through a greater emphasis on general health

and mental and physical fitness as the means of achieving resilience (de Kloet et al. 2005; McEwen et al. 2015; McEwen and Akil 2020). Thus, a primary goal should be to create incentives at home and in the workplace that encourage individuals to learn tools that will help them engage in beneficial lifestyle practices.

In this context, there is a general interest in developing methods for stress assessment and monitoring, especially at the workplace (Carneiro et al. 2019). Indeed, while occupational stress affects individuals personally, it also has a severe economic impact. This substantial financial burden calls for the development and implementation of initiatives for stress management that would simultaneously reduce the enormous costs associated with absenteeism and presenteeism and improve employee overall physical and mental well-being. Moreover, the primary aim of these interventions would be that both people and businesses can not just survive but thrive in the global economy associated with constant VUCA (Volatility, Uncertainty, Complexity, and Ambiguity) due to frequent economic, ecological, and pandemic crisis. The most recent example pertains to the pandemic caused by the COVID-19 virus, which led to transitioning to a "*New Normal*" workplace and industry practices, forcing most companies to shift to remote, home-based working and online trade.

Meanwhile, recent technological and scientific advances in organizational psychology related to workplace stress management and monitoring added several innovative appliances and methods to the traditional ones (e.g., physiological sensors, specific job-tailored questionnaires). The most progressive approach uses various ambient intelligence systems to continuously monitor employee well-being and stress levels in the working environments without interference. These systems should then be adjusted and tailored according to the corporate environment and cultural specifics. They would also include identifying the critical stressors related to the specific job design and task, followed by a detailed evaluation of their impact on the employee's work performance and job satisfaction levels, eventually enabling successful implementation of long-term personalized workplace stress management practices.

Indeed, in addition to detecting and monitoring stress, some workplace studies also proposed various modalities that could be easily employed to alleviate stress. For example, some ancient techniques found to be highly beneficial in managing stress are yoga and meditation, which could be performed during regular breaks in indoor or outdoor corporate facilities. An even better and more efficient alternative would be the introduction of "*just in time*" effective stress management interventions to alleviate physical and mental tension associated with increased stress levels "*on-the-spot*" without leaving the office space, thus assuring continuous and long-lasting personal well-being, which would eventually translate into improved employee engagement and increased performance (Can et al. 2019).

Finally, all these developments gave a solid impetus to human resource professionals to improve or recreate policies related to mental health promotion while assuring continuous education and training of leaders, managers, and employees in various occupational and psychological safety practices leading to increased workplace and subjective well-being.

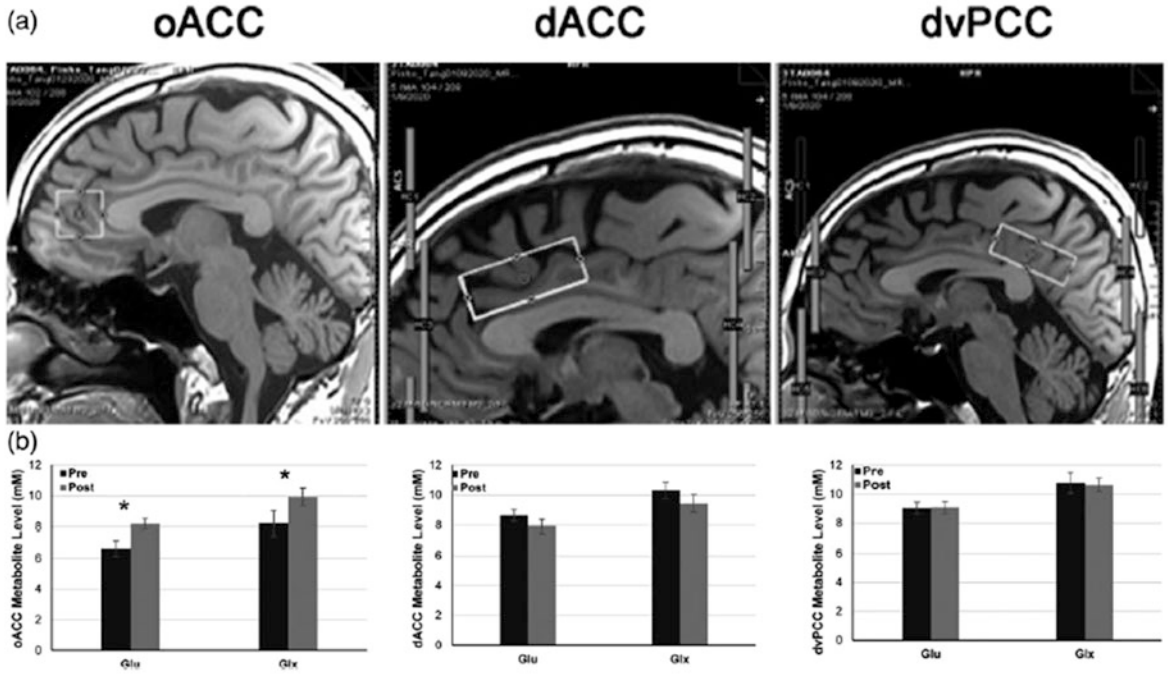
Although developing such innovative systems for monitoring and managing workplace stress is challenging, there are even more issues to deal with when taking a step outside controlled environments. Typically, researchers aim to detect stress levels and help all individuals to better cope with high-stress levels in their daily lives. However, this approach is associated with multiple deficiencies in stress-detection accuracy and reliability. Indeed, most of these assessment methods rely on user reports, surveys, and non-validated questionnaires, which could lead stress management and health experts in the wrong direction due to their high levels of subjectivity.

Novel methodologies involving integrating different physiological measures are required to improve stress-detection accuracy, with the final goal to bring benefits to the general population. However, designing and bringing such applications to mass markets will strongly depend on progress in technology, data analysis, and evaluation methodologies to increase the comfort and accuracy of stress measurement. Moreover, high inter-individual variability in results obtained would pose significant difficulties to their accurate interpretation and negatively impact appropriate designing and implementation of the specific stress management intervention on a community level.

Beyond external interventions, internal factors or traits may also be amplified and targeted to increase individual coping strategies and prevent associated adverse psychological and physical outcomes related to chronic stress exposure. Indeed, individuals perceive stressful events differently, and some individuals are less vulnerable to stress than others, which are deemed resilient. Since most people who experience stressful events do not develop psychopathology, increasing interest has been directed towards potential psychological resilience-promoting factors that may be associated with or influencing mental toughness. Targeting these factors with newly developed therapeutics may be one of the modalities to decrease the incidence of mental and systemic illnesses.

It has been shown that increasing resiliency throughout the lifespan can slow down the aging process, improve general health, and protect against external and internal stressors. Regrettably, most researchers centered their studies on creating resilience measurement instruments and identifying mental and physical factors associated with psychological resilience rather than developing and testing effective resilience interventions and therapeutic solutions (McEwen 2016). However, the minority of them focused on assessing the effects of various ancient and novel individual and psychosocial interventions that would increase individual coping mechanisms. For example, mindfulness training, a cornerstone of Buddhist practice, has been consistently related to improved concentration, increased self-awareness, and decreased mind wandering resulting in positive mood states and more efficient emotion regulation.

Moreover, Tang et al. showed for the first time that regular brief mindfulness practice mediated beneficial effects on brain activity, morphology, and functional connectivity through changes in brain glutamate metabolism in the ACC (see Fig. 4.4) (Tang et al. 2020).



(a) Regions of interest (ROIs) in ^{1}H-MRS. Voxel positioning is shown on structural MR images for three regions of interest (ROIs). The voxel size was $20 \times 20 \times 20\,mm^3$ for rostral ACC (oACC), and $40 \times 15 \times 15\,mm^3$ for dorsal-ventral PCC (dvPCC) and dorsal ACC (dACC). (b) Glutamate and Glx concentrations in three ROIs before and after IBMT. Glutamate (Glu) and Glx (glutamate and glutamine) concentrations (mM) in three brain regions (oACC, dvPCC, and dACC) following 10 h IBMT.

Fig. 4.4 Magnetic Resonance Spectroscopy (MRS) results indicating a significant increase in glutamate and Glx (glutamate + glutamine) metabolism in the ACC after 10 h of practicing the integrative body-mind training (IBMT). According to the authors, these imaging results raise the possibility that engaging in mindfulness practices could prevent and ameliorate age-related cognitive decline through functional and structural changes in the ACC (Tang et al. 2020)

In contrast to psychological interventions, the knowledge about the possibility of enhancing mind and body resilience with pharmaceuticals is still scarce and lacks human clinical trial data. Moreover, increased insights about the significant challenges and impediments associated with real-world implementation of established interventions such as intermittent fasting and physical exercise noted in recent community trials additionally discouraged neuroscientists and clinicians from diligently pursuing studies on resilience (van der Kooij 2020). In contrast, in the last couple of decades, sustained efforts from industry members mainly concentrated on developing drugs and nutraceuticals that would efficiently increase mental and physical resilience without adopting specific long-term lifestyle modifications. An excellent example is a recent repurposing of medications with mTOR inhibitory properties, such as Rapamycin (immunosuppressant), Resveratrol (natural polyphenolic compound), and Metformin (antidiabetic drug) into the category of "caloric restriction mimetics."

Unfortunately, these pioneering activities were not too rewarding for their initiators and promoters as most proof-of-concept studies, clinical development trials, and data obtained during post-marketing surveillance showed mixed and inconsistent effects of these compounds on stress biomarkers and chronic stress or specific stress-related condition symptom levels (Faye et al. 2018).

Developing the most sophisticated stress-prevention modalities like innovative drugs with pro-resilient properties would require a long-term collaborative strategical commitment of both academia and industry representatives, including their significant joint workforce, time, and financial investments.

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Chapter 5
The Role of the Glutamate System in Posttraumatic Stress Disorder and Glutamate-Based Treatments

M. Popoli, A. Ieraci, and L. Musazzi

Abstract Posttraumatic Stress Disorder (PTSD) is a chronic mental illness with limited options for treatment, characterized by intrusive memory of trauma, avoidance, hyperarousal, emotional numbing, and anhedonia. PTSD is often triggered by exposure to a single traumatic experience, with high prevalence among war veterans. PTSD, together with other neuropsychiatric disorders, involves long-term changes in the structure and function of brain areas, synaptic disconnection, and changes in large-scale brain networks.

Trauma-focused psychotherapy is considered first-line treatment, with greater and more persistent efficacy than pharmacotherapeutic approaches. Pharmacological treatments include SSRI, particularly paroxetine and sertraline, and other traditional antidepressants. The effect size of efficacy for these drugs is often small, with high treatment resistance in certain populations. In recent years a major shift in the conceptual framework of neuropsychiatric disorders has occurred, from the monoamine hypothesis to a neuroplasticity hypothesis, in which the glutamate system is conceived as a primary mediator of pathology and a straight target for antidepressant drugs. Novel potential treatment options have emerged in recent years, in particular several modulators of the glutamate system, including ketamine, riluzole, D-cycloserine, *N*-acetylcysteine. Ketamine, the prototypical rapid-acting antidepressant, has received much attention for its complex mechanism of action (including clinical trials), and has been proposed as a prophylactic agent against the onset of PTSD after exposure to traumatic stress.

This chapter will explore new pharmacological approaches to the therapy of PTSD, based on the modulation of the glutamate system.

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_5

Keywords PTSD · Glutamate · Stress disorders · Neuroplasticity · Synaptic morphology · Ketamine · Fear memory

5.1 Posttraumatic Stress Disorder: A Stress-Related Disorder

Most brain disorders are characterized by the so-called gene–environment interaction, where the presence of mutations in the genome (particularly single-nucleotide polymorphisms) moderates the effects of environmental factors in relation to resilience or vulnerability for different psychopathologies. The most common and influential among environmental factors in neuropsychiatric disorders are various adverse life events, often conveyed under the name of "stress." While it is known that stress is a primary risk factor for neuropsychiatric disorders, and the popular use gives the word "stress" a negative connotation, it is important to remember that the stress response is a physiological reaction that subserves the adaptation to changes in the environment (including those that are, or may seem, menacing). Under this respect, it is not just the nature of the stressor itself but rather the kind of response that may generate deleterious consequences for health (Duman and Aghajanian 2012; Pitman et al. 2012; Popoli et al. 2012; McEwen et al. 2015; Duman et al. 2016; Musazzi et al. 2017). A pro-adaptive stress response facilitates the achievement of a new level of adaptation through changes (*allostatic change*), while a maladaptive response sets the system off-balance favoring the onset of psychopathology (*allostatic overload*) (McEwen 2017).

However, in Posttraumatic Stress Disorder (PTSD), a debilitating, often chronic and comorbid, mental illness with as yet limited options for treatment, the relationship with the exposure to strong, traumatic events is particularly evident. It has been estimated that 70% of the world population has been exposed to trauma and approximately 6% of trauma-exposed individuals develop PTSD, although the prevalence of PTSD varies widely across studies, due to different assessment methods, kinds of stress and populations. However, many studies agree that its prevalence is lower in the general population compared, for instance, with war veterans (25%), suggesting that stressful events have a key role in its pathophysiology (Koenen et al. 2017; Fulton et al. 2015). Typical symptoms of PTSD include intrusive memories of trauma, avoidance of trauma-related stimuli, increased arousal, vigilance and irritability, negative cognition and mood (including anhedonia) (Fig. 5.1) (Kessler et al. 1995; APA 2013).

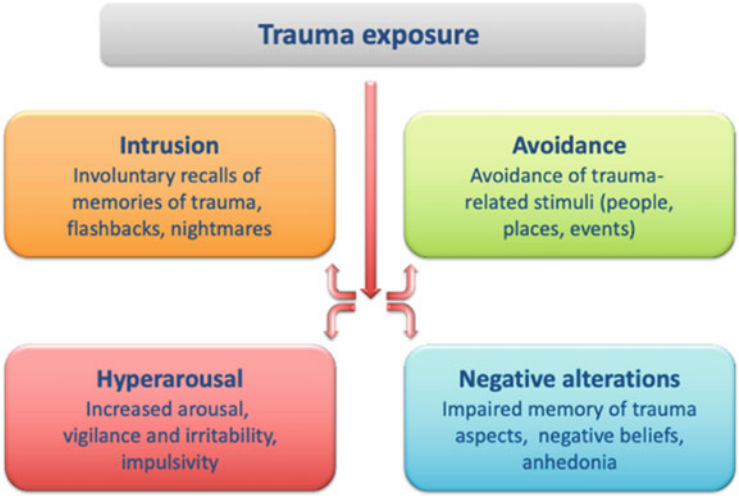

Fig. 5.1 Core symptoms of Posttraumatic Stress Disorders

5.1.1 Posttraumatic Stress Disorder Involves Dysfunctional Processing of Fear Memory

PTSD is conceptualized as a disorder involving dysfunctional processing of fear and fear memory. Fear is a primary emotion that has obvious evolutionary value, defending our biological integrity from actual or envisaged danger, and may predispose to a fight-or-flight reaction (Popoli et al. 2012; LeDoux 2014; McEwen et al. 2015; McEwen 2017). The biological mechanisms of fear memory have been extensively studied in rodent models, particularly by using fear conditioning protocols (Milad and Quirk 2012; Izquierdo et al. 2016). In a classical protocol, rodents receive brief inescapable shocks (unconditioned stimulus; US) coupled with an acoustic or visual stimulus (conditioned stimulus; CS). The phase following this training is called "fear memory consolidation." When they are placed again in the same context and exposed to CS (in the absence of US) they will freeze because they associate CS to US. When this is repeated over time, rodents will learn that the CS is no more painful and extinguish the fear memory. This phase is called "fear memory extinction." In early studies, it was proposed that fear conditioning may serve as an animal model for anxiety disorders, including PTSD, and could be useful for understanding the biological mechanisms of human disorders. PTSD-affected subjects may form strong associations between traumatic events and sensory cues present in the context of the trauma, which at some point become resistant to extinction (Milad and Quirk 2012). Rodent studies showed that both fear consolidation and fear extinction are active forms of learning, requiring (among others) *N*-methyl-D-aspartate (NMDA) receptors for glutamate. Both rodent studies and clinical studies with functional Magnetic Resonance Imaging (fMRI) identified a

fear circuitry involving (among others) amygdala and prefrontal cortex (PFC) as key structures responsible for the formation and processing of fear memory. Activation of amygdala is required for acquisition and expression of conditioned fear responses, while PFC is activated in top-down control of amygdala and fear memory processing. In particular, a number of studies showed that the rodent prelimbic (PL) cortex increases fear expression and inhibits extinction, while infralimbic (IL) cortex inhibits fear expression and increases extinction. In humans, fMRI studies showed that dorsal anterior cingulate cortex (dACC) and ventromedial PFC (vmPFC) are homologous of PL and IL cortex in rodents, respectively, in relation to modulation of fear expression (Milad and Quirk 2012). In recent years, the discovery of large-scale human brain networks has added a new level of complexity to pathophysiology of PTSD (see Sect. 5.4.1, below).

The fear extinction mechanism has a clear translational value as a model for the exposure therapy, widely used in the treatment of PTSD. A prominent feature of exposure therapy is the extinction of the memory that caused the trauma, often a fear memory that keeps coming back in the presence of environmental cues. The objective of exposure therapy is to attain a degree of extinction by making the subject re-experience elements of the traumatic events, through remembering the events and engaging with, rather than avoiding, reminders of the trauma.

5.1.2 Posttraumatic Stress Disorder and Acute Stressors

In many cases, the exposure to a single traumatic event (a traffic accident, a natural catastrophe, an episode of violence) is enough to trigger PTSD later. When humans are exposed to trauma many experience symptoms typical of PTSD in the following days and weeks. However, most of them recover and this is considered a form of pro-adaptive stress response (*allostatic change*) and a manifestation of resilience. Instead, a minor proportion of exposed subjects develop a permanent disorder that may last for years or even a lifetime (Abdallah et al. 2019). These subjects show a long-term maladaptive stress response (*allostatic overload*), a manifestation of vulnerability. The difference between resilience and vulnerability is presumably due to a complex interaction of genetic signature, sex, previous life events, and severity of trauma. At least in PTSD, this last factor seems quite important because the risk of developing the disorder is proportional to severity of the stressor. This makes the odds even more difficult to calculate, because implies for all subjects an individual threshold, separating resilience from vulnerability toward the consequences of trauma. Paraphrasing the notorious line "every man has his price," one could say that every human has his/her stress threshold. Furthermore, for some reason we do not understand, the percentage of subjects developing PTSD is higher after trauma linked to human-related technology (car accident, plane crash, etc.) than to natural causes (earthquakes, etc.).

It is common, in theoretical frameworks of mental illness, to consider chronic stress as a major risk factor in pathophysiology. Yet, we know that at least for PTSD

the disorder can be triggered by a single, although traumatic, stressful event. This suggests that in some way even acute stress (lasting minutes to hours) can have long-lasting consequences (years), perhaps by establishing a prolonged maladaptive stress response. Along with other mediators, the glutamate system has a central role in this maladaptive response, as shown in the following sections.

5.2 Central Role of the Glutamate System in Pathophysiology of Posttraumatic Stress Disorder

Glutamate is by far the most abundant neurotransmitter in the brain, although its role was not recognized until the early 1980s, much later than the monoaminergic neurotransmitters (Sanacora et al. 2012). Particularly in the neocortex, about 80% of all neurons release glutamate and form about 85% of all synapses (Orrego and Villanueva 1993; Douglas and Martin 2007; Nava et al. 2015). Most of the remaining neurons, about 20%, are γ-aminobutyric acid (GABA)-ergic inhibitory interneurons, forming about 15% of synapses; all other synapses, including monoaminergic, acetylcholinergic, peptidergic or using other mediators, represent a much smaller proportion. As an example, in the whole brain, there are approximately two to three hundred thousand serotonergic neurons, compared to the roughly hundred billion total neurons (Baker et al. 1991). This pervasive presence of glutamate is not without a cost. The concentration of glutamate in the brain tissue is quite high (up to 10 mM), but the extracellular concentration is about ten thousand times lower (<1 μM), thanks to the presence of efficient transporters on the membranes of both astrocytes and neurons. If this mechanism is impaired, like in brain ischemia or trauma, higher levels of extracellular glutamate become excitotoxic and may induce neuronal death.

Throughout the years a major shift in the conceptual framework investigating pathophysiology and treatment of neuropsychiatric disorders has occurred, from a mainly monoamine-oriented hypothesis to a neuroplasticity hypothesis, in which the role of the glutamate system is conceived as a primary mediator of neuropsychiatric pathology and also a straight target for antidepressant drugs (Sanacora et al. 2012; Duman and Aghajanian 2012; Abdallah et al. 2015; Lener et al. 2017; Murrough et al. 2017). This has not undermined the important role of monoaminergic neurotransmitters in the fine-tuning of cognitive and emotional functions, and in the pathophysiology of neuropsychiatric disorders.

There is a large and consistent body of evidence in the literature clearly showing dysregulation of the glutamate system in stress rodent models and in human neuropsychiatric disorders, particularly in PTSD, as illustrated below.

5.3 Preclinical Evidence of Dysregulation of Glutamatergic System in Posttraumatic Stress Disorder: Rodent Stress Models

A number of studies in recent years analyzed in detail the role of stress and stress-related molecular/cellular/functional effects in the glutamate system, with regard to pathophysiology of neuropsychiatric disorders (Gorman and Docherty 2010; Duman and Aghajanian 2012; Popoli et al. 2012; Sanacora et al. 2012; McEwen et al. 2015; Averill et al. 2017; Musazzi et al. 2017). Within this framework, a large body of evidence on the role of the glutamate system comes from repeated or chronic stress studies in rodents. Now, while it is generally assumed that chronic stress is associated with the pathogenesis of mood and anxiety disorders, the fact that PTSD may also be triggered by acute exposure to a traumatic stressor contrasts with this assumption. Indeed, it has been suggested that essential requirements for animal models of PTSD should include the following: (1) the trauma must be relatively severe, (2) a short duration of protocol should be sufficient to provoke PTSD-like symptoms, (3) the intensity of the trauma should predict the severity of outcome, and (4) significant interindividual variability should be observed in outcomes (Siegmund and Wotjak 2006). For a comprehensive classification of the different animal models of PTSD, see Flandreau and Toth (2018). Therefore, it seems essential, in rodent models of PTSD, to assess long-term cellular/molecular changes that are associated to PTSD-like long-term outcomes, possibly induced by short-term exposure to traumatic stress (Flandreau and Toth 2018; Musazzi et al. 2018).

5.3.1 Mechanism of Neuroarchitecture Changes in Rodent Stress Models

The most consistently observed consequences of chronic stress in rodents are the changes of neuroarchitecture in three brain areas: prefrontal cortex (PFC), hippocampus (HPC), and amygdala. A whole array of studies using different stress protocols showed that chronic stress application reduces apical dendrite length and branching of medial PFC pyramidal neurons (layers II/III and V) and HPC CA3 pyramidal neurons, while increasing dendritic density in basolateral amygdala. In general, this dendritic atrophy in PFC and HPC goes along with a reduction of the density of synaptic spines, and suggests that chronic stress induces a "synaptic disconnection" syndrome within and between these areas (Duman and Aghajanian 2012; Sousa and Almeida 2012; Musazzi et al. 2017; Duman et al. 2019; Tornese et al. 2019a). The preclinical results over the years have integrated, and contributed to explain, the clinical neuroimaging data showing shrinkage of PFC and HPC, particularly in major depression (see Sect. 5.4 below). What these studies compellingly showed is the central role of the glutamate system in pathophysiology,

because most neurons and synapses in these areas are glutamatergic, in particular pyramidal neurons displaying synaptic disconnection.

A likely reason for these neuroarchitecture changes has been found in the glutamate system itself, and is intimately linked to the mechanism of stress response and its major mediators glucocorticoids. Early microdialysis found that different acute stress protocols or administration of corticosterone (CORT; the main glucocorticoid stress hormone in rodents) rapidly increases the level of extracellular glutamate in PFC and HPC (Bagley and Moghaddam 1997; Lowy et al. 1993; Moghaddam 1993; Venero and Borrell 1999). However, total extracellular glutamate does not exactly correspond to the pool of glutamate that is actively released at synapses, and the actual demonstration of the impact of stress on glutamate release was given by methods that measure the exocytotic release of aminoacid neurotransmitters (Sanacora et al. 2012). In particular, the action of acute inescapable footshock stress (FS) on glutamate release in PFC was shown by measuring glutamate release from superfused purified synaptic terminals (synaptosomes), a method that allows accurate characterization of the presynaptic release of endogenous amino acid neurotransmitters (Popoli et al. 2012). The FS protocol induces long-term behavioral changes resembling PTSD, and has the advantage to allow precise and reproducible control of the shock parameters, as well as clearly defined and reproducible context for stress. Long-term behavioral changes after FS include social avoidance, defensive behavior, hypervigilance, sleep disturbances, generalization of fear (Bali and Jaggi 2015; Flandreau and Toth 2018).

It was found that acute FS rapidly enhanced depolarization-evoked release of endogenous glutamate from PFC synaptosomes, soon after completion of the stress protocol (40 min). The enhancement of glutamate release was dependent on elevation of CORT levels and binding to CORT receptors, because treatment with selective antagonists of glucocorticoid or mineralocorticoid receptor (GR/MR) prevented the release increase. The enhancement of glutamate release was confirmed by patch-clamp recordings in PFC and was prevented by chronic treatment with different antidepressants (Musazzi et al. 2010). The mechanism was further dissected by showing that CORT binds GR/MR located at glutamate synapses and induces rapid (nongenomic) enhancement of the trafficking of glutamate synaptic vesicles into the readily releasable pool (RRP), dependent on phosphorylation of Ser^{9} in synapsin I (Treccani et al. 2014). These studies for the first time clearly showed that CORT directly enhances presynaptic release of glutamate via neuronal membrane-associated receptors. The outcome of this stress-induced surge of glutamate is probably amplified by malfunction of glutamate clearance by glial transporters, although the evidence for this after acute stress is scanty and limited to the HPC (Yang et al. 2005; Homiack et al. 2018). Moreover, ^{13}C-Magnetic Resonance Spectroscopy studies demonstrated that chronic stress exposure decreases the cycling and metabolism of glutamate and glutamine in PFC, an effect attributed to a reduction in glial metabolism (Banasr et al. 2010; Duman et al. 2019).

In line with these findings, a widely shared hypothesis suggests that abnormal enhancement of glutamate release induced by stress/CORT results in dendritic atrophy, reduced spine density, and synaptic disconnection in PFC and HPC,

when stress is repeated or sustained over time (Duman and Aghajanian 2012; Sanacora et al. 2012; Abdallah et al. 2015; McEwen et al. 2015; Musazzi et al. 2017; Duman et al. 2019). Consistent with the hypothesis, blockade of *N*-methyl-D-aspartate (NMDA) receptors during repeated restraint stress prevented stress-induced apical dendritic retraction in mPFC (Martin and Wellman 2011). Initially, retraction of apical dendrites could be an adaptive change against the glutamate surge and spillover; for a discussion, see Musazzi et al. (2017).

5.3.2 *Long-term Maladaptive Stress Response After Acute Stress Involves the Glutamate System*

As addressed in Sect. 5.3.1, neuroarchitecture changes in PFC and HPC have been observed in many rodent studies using chronic protocols of stress; these findings have contributed supporting the assumption that chronic stress is a major factor in the induction of structural brain changes. However, in the last several years a few studies have investigated the outcome of a single exposure to stress or of a few closely spaced episodes of stress on neuroarchitecture. Interestingly, although with different modalities, these studies found that also acute stress may induce atrophy of apical dendrites or loss of synaptic spines in HPC/PFC. First, Izquierdo et al. (2006) showed that a brief session of forced swim stress administered each day for 3 days, followed by fear conditioning and extinction sessions, induced atrophy of apical dendrites in infralimbic pyramidal neurons of medial PFC, together with impairment of fear extinction. In the same study, it was shown that even a single session of swim stress was enough to induce the dendrite atrophy observed at the end of the experiment. Second, Hajszan et al. (2009) found that rats exposed to inescapable FS in the context of a learned helplessness protocol (a popular model of depression) showed similar loss of synaptic spines in CA1, CA3 and dentate gyrus of hippocampus, whether the spine density was assessed 1 day or 7 days after FS. A single injection of corticosterone reproduced both the hippocampal neuroarchitecture changes and the behavioral responses induced by inescapable FS. Third, Chen et al. (2010) investigated the effects of short multimodal stress, a protocol containing different forms of stress that lasts 5 h. They found reduction of synaptic spines density in hippocampal CA3 area within hours after cessation of stress, along with impairment of long-term potentiation, a well-known form of synaptic plasticity. Taken together, these findings suggest that a single stressful event with short duration or a few events closely spaced in time may induce rapid neuroarchitecture changes in the brain.

Recently, using the same acute FS protocol that allowed a thorough dissection of the action of stress and CORT on glutamate release in PFC (see above), it was found that this brief exposure to stress induces after 24 h significant atrophy of apical dendrites in pyramidal neurons of prelimbic PFC, similar to changes found by studies using chronic stress protocols. Unexpectedly, this rapid change in

neuroarchitecture was also found to be a stable long-term change, because it was observed 1 and 2 weeks after acute FS (Nava et al. 2017). In parallel, it was found that, different from the CORT elevation that was back to normal level soon after cessation of stress, depolarization-evoked glutamate release was enhanced at all times assessed up to at least 24 h. The RRP of glutamate vesicles (largely responsible for the enhancement of release) and the phosphorylation of Ser^9 of synapsin I, previously shown to be functional to the RRP size increase, were also increased at all times including 24 h (Musazzi et al. 2016). Taken together, these last studies showed that acute stress exerts rapid, destabilizing effects on synaptic glutamate in PFC, as well as sustained enhancement of depolarization-dependent release of glutamate, at least for 24 h. During this time, between start of stress exposure and 24 h, rapid atrophy of apical dendrites occurs in PL PFC; this atrophy is not transient but lasts for at least 2 weeks. Therefore, for the first time, it was clearly shown that a brief episode of inescapable stress may induce long-term neuroarchitecture changes in the brain, with synaptic disconnection and likely functional impairment. Indeed, impairment in T-maze performance, a test for working memory (largely dependent on PFC), was observed in the rats 24 h after FS stress (Musazzi et al. 2019).

Overall, the few studies that assessed the effects of acute or sub-acute stress on neuroarchitecture demonstrated that brief exposure to stress may induce not only rapid changes (within hours) but also reproduce the sustained structural changes that are typical of chronic stress. These results and future developments may help understand how acute FS and other protocols used as models of PTSD may induce long-term behavioral changes. An interesting consequence of this is that protocols based on acute stress models could work somewhat better than chronic models to understand not only pathophysiology of PTSD, but also of depression and other stress-related disorders. Indeed, when chronic stress models are used, we look at the outcome after several days or weeks of repeated stress, just missing everything that happened in the middle. Instead, when using acute stress protocols it is easier to follow the changes with time in structural/functional readouts, along with changes in behavioral readouts, something that could tell us more about relevant pathophysiological changes. An added value of this approach is that this is also an excellent ground for testing novel drugs, such as the new rapid-acting antidepressants (Musazzi et al. 2018).

5.4 Clinical Evidence of Dysregulation of Glutamatergic System in Posttraumatic Stress Disorder: Neuroimaging Studies

Clinical evidence for dysregulation of glutamatergic system in neuropsychiatric disorders comes from a large array of neuroimaging studies, which explored over the last three decades the brain structural and functional changes associated with mental illness (Koolschijn et al. 2009; Kempton et al. 2011; Sanacora et al. 2012;

Schmaal et al. 2016). All the cortical and limbic areas where changes have been found are areas where glutamate neurons and synapses predominate (see Sect. 5.2). Some of these areas, in particular HPC and PFC are smaller in major depression (the largest number of studies) and, in a few studies, a reduced density of dendrites and synaptic spines has also been found in the same areas, like in preclinical studies (see Sect. 5.3.1) (Soetanto et al. 2010; Kang et al. 2012). Therefore, it is assumed that, in both rodents and humans, the neuroarchitecture changes are the main reason for the volumetric changes described by neuroimaging (Musazzi et al. 2017). Within this body of work a fair number of published works have described the relevant changes in PTSD brain, with many studies using structural or functional Magnetic Resonance Imaging (MRI). Main areas implicated in PTSD by the findings from MRI studies include the HPC, mPFC, dorsal anterior cingulate cortex (dACC), insula and amygdala. In particular, structural MRI studies found lower volume of HPC, rostral ventromedial PFC, and dACC in PTSD (Bremner et al. 1995; Gurvits et al. 1996; Smith 2005; Kitayama et al. 2006; Kasai et al. 2008). A recent meta-analysis evaluated a number of studies, comparing PTSD with control groups that included non-traumatized and traumatized subjects in the analysis. The results showed significant volumetric reduction in various areas (Fig. 5.2) and also that PTSD patients exhibited reduced total brain and intracranial volume. As many of the areas showing volumetric changes in PTSD were the same as in major depression but total brain volume was not changed in the latter, the changes in total brain volume seem to distinguish PTSD from depression (Bromis et al. 2018).

Functional MRI or Positron Emission Tomography (PET) has been used in most functional neuroimaging studies. They found altered activity in amygdala, vmPFC, dACC, HPC, and insula. Amygdala (which is powerfully activated by stressful events and has a crucial role in fear learning), dACC, and insula were found to be hyperactivated in PTSD. Instead, vmPFC showed reduced activation, while HPC showed less or more activation in different studies. Decreased vmPFC activity was associated with increased amygdala activity. Many of these studies (both structural and functional) have been the object of meta-analyses, substantially confirming the alterations found (Karl et al. 2006; Kitayama et al. 2006; Hayes et al. 2012; O'Doherty et al. 2015; Bromis et al. 2018; Kunimatsu et al. 2019). These findings are in line with a classical network model of PTSD, which posits a reduced capacity of cortical areas to control fear and negative emotional responses, with increased attentional bias toward threat, increased fear response, and defective extinction of traumatic memories. In this theoretical framework, the capability of HPC to use contextual cues to signal safety, and of vmPFC to promote extinction of conditioned emotional responses related to traumatic learning, would be impaired (Rauch et al. 2006; Pitman et al. 2012).

With regard to neuroimaging data related to other neuropsychiatric disorders (e.g., depression) it has been much debated if volumetric reductions in HPC and PFC are a result of a maladaptive stress response following exposure to trauma or rather represent a pre-existing risk factor, due to genetic signature and/or previous life events. The same applies to volumetric changes observed in PTSD. A study with monozygotic twins, in which one of the twins was a war veteran with PTSD and the

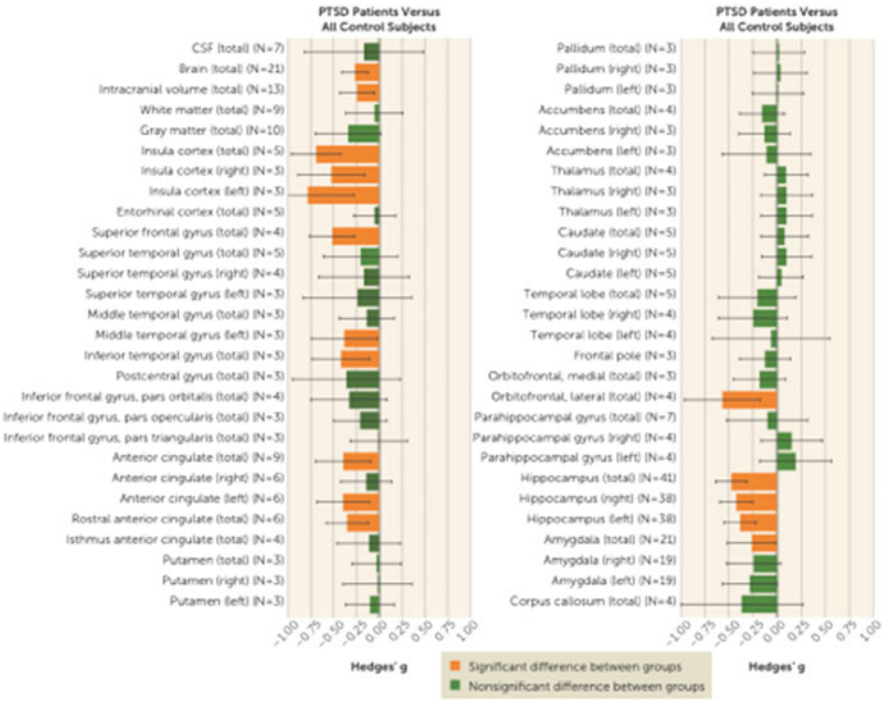

Fig. 5.2 Results from a Region-of-Interest meta-analysis of 66 structural MRI studies comparing patients with PTSD with non-traumatized or traumatized control subjects. Compared with all control subjects, PTSD patients had reduced brain volume, intracranial volume, and reduced volumes of the hippocampus, insula, and anterior cingulate. Hedges' g (Cohen effect size with small-sample correction) is shown for each structure, with 95% confidence intervals. The effect size is positive when the structure is larger in patients with PTSD compared with control subjects and negative when the structure is smaller in PTSD patients. The number of studies included in each meta-analysis is indicated for each structure (from Bromis et al. 2018)

other had not been exposed to combat, failed to find HPC volume difference among the twins. In both twins HPC was smaller than in veterans that did not develop PTSD. These results suggested that smaller HPC volume represents a pre-existing vulnerability factor (Gilbertson et al. 2002). On the other hand, other studies showed that functional measures, including skin conductance response and heart-rate response to loud tone stimuli, are acquired features of the disorder (Orr et al. 2003; Metzger et al. 2008; Milad et al. 2008). Overall, a meta-analysis of 39 studies investigating HPC volume in subjects with PTSD, trauma-exposed controls without PTSD, and trauma-unexposed controls, found that trauma exposure itself, in the absence of PTSD, was associated with HPC volume deficits, with further reduction in subjects with PTSD (Woon et al. 2010). This would suggest that reduced HPC volume is a consequence of trauma and not a pre-existing risk factor. For a discussion, see Pitman et al. (2012).

5.4.1 *Clinical Evidence of Dysregulation of Glutamatergic System in Posttraumatic Stress Disorder: Network-Based Models*

As addressed above, there is compelling evidence for structural and functional brain network dysfunction in PTSD, traditionally based on abnormal connection between HPC, PFC, and amygdala. In recent years, based on a large body of evidence from neuroimaging studies, a more complex model has been proposed, the so-called triple network model, which allows a broad synthesis from a system's neuroscience perspective (Menon 2011). The model stems from the discovery that the human brain is intrinsically organized into coherent large-scale functional networks. The three networks, that have been identified across a wide range of cognitive tasks, are: the Central Executive Network (CEN), the Default-Mode Network (DMN), and the Salience Network (SN). The CEN, also called frontoparietal system, is anchored in dorsolateral PFC and posterior parietal cortex (PPC). This network is responsible for high-level cognitive functions such as planning, decision making, and the control of attention and working memory; it is engaged in goal-directed behavior and in top-down regulation of emotions. The DMN spans various areas, including the PPC, medial PFC, and medial temporal lobe, including HPC. Contrary to the CEN, the DMN is typically deactivated during most stimulus-driven cognitive tasks. It is a large-scale network of brain areas forming an integrated system for self-related cognitive activity, including autobiographical, self-monitoring, and social functions. The SN is a large-scale brain network, anchored in the dorsal anterior cingulate cortex (dACC) and frontoinsular cortex (FIC), which also includes the amygdala and the substantia nigra/ventral tegmental area. It is involved in detecting, integrating, and filtering relevant interoceptive, autonomic, and emotional information. It is important for detection and mapping of salient external inputs and internal events; it has been suggested that a key function of this network is to identify the most homeostatically relevant among different internal and external stimuli to guide behavior (Menon 2011 and refs. therein).

CEN and SN are typically activated during stimulus-driven processing of cognitive and affective information, while DMN shows reduced activation during tasks in which self-referential and stimulus-independent memory recall is not crucial. The triple network model proposes that deficits in engagement and disengagement of these three core networks play a significant role in both neuropsychiatric and neurological disorders. A key role in the model is assigned to the SN, which may show weak salience detection and mapping of goal-relevant external stimuli and internal mental events. The defective action of SN may give rise to aberrant engagement of the frontoparietal CEN, compromising cognition and goal-relevant adaptive behavior. Also, weak engagement or disengagement of the DMN by salient events is associated with altered self-referential mental activity, such as excessive rumination in depressed patients. It was proposed that aberrant access, engagement, and disengagement of these three large-scale neurocognitive networks play a prominent role in several brain disorders, including schizophrenia, depression, anxiety, dementia, and autism.

This theoretical framework integrates on a higher level of complexity the vast literature on neuroarchitecture changes revealed by neuroimaging and poses several questions that are still open. These questions include the following: how are the neuroarchitecture changes in different areas associated to specific functional network changes and what are the specific changes (if any) relevant for different symptomatology? For instance, the evidence for altered glutamatergic metabolism within these networks in major depression has given further insight into how aberrant nodes influence within- and across-network interactions, and how this is linked to a chore symptom of depression, e.g., anhedonia (Walter et al. 2009; Horn et al. 2010).

Recently, the triple network model framework has been applied to PTSD pathophysiology (Fig. 5.3) (Akiki et al. 2017). Emphasizing the central role of the SN, it

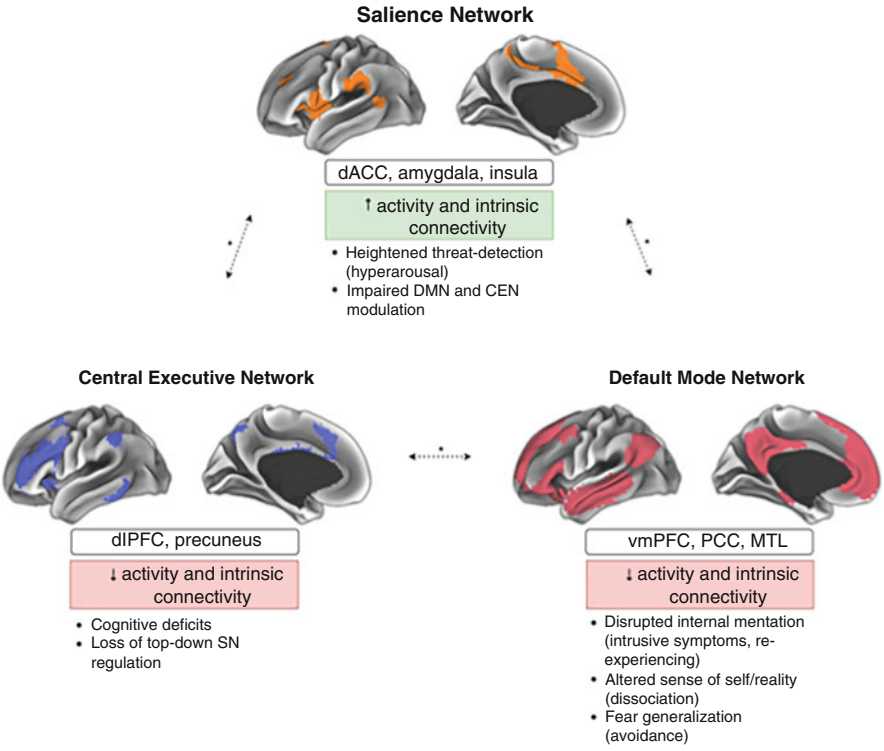

Fig. 5.3 Network-based neurobiological model of PTSD. The salience network (SN; orange), the central executive network (CEN; blue), and the default mode network (DMN; red) are represented. Under each network the notable regions of interest, the putative changes in activity and connectivity in PTSD and the resulting behavioral abnormalities are reported. According to the model, the SN is hyperconnected/hyperactive, has a low threshold for perceived saliency (underlying hyperarousal), and exerts aberrant modulation of DMN-CEN. The CEN is weakly interconnected/hypoactive (underlying impaired cognition), resulting in impaired top-down SN regulation. The DMN is weakly interconnected/hypoactive, resulting in impaired ability to maintain a homeostatic inner state (intrusive symptoms), altered sense of self/reality (dissociation), and fear generalization (avoidance) (from Akiki et al. 2017)

has been proposed that hyperactivity of SN, with a low threshold for perceived saliency, is involved in hyperarousal symptoms and heightened threat detection. This in turn brings about aberrant modulation of CEN and DMN (e.g., switching between task-relevant and task-irrelevant behavior). Defective engagement of CEN results in cognitive deficits and loss of top-down regulation of SN. Defective engagement of DMN results in disrupted ability to maintain a homeostatic inner state (e.g., intrusive symptoms, re-experiencing), altered sense of self/reality (dissociation), and fear generalization (avoidance). Based on MRI studies of PTSD, it has been suggested that changes in the SN network may predict psychotherapy treatment response using exposure therapies. Successful psychotherapy treatment response would involve the ability to downregulate amygdala activity to trauma-related stimuli, through modulation of the SN (Szeszko and Yehuda 2019).

It is not clear yet how the structural changes observed in the different areas in PTSD (see Sect. 5.4) are related with the connectivity changes within and across networks. Also, it is not clear if the structural changes are a causal factor for the functional abnormalities, or whether the functional abnormalities induce long-term structural changes. Longitudinal studies are necessary to clarify these points. However, once again, it should be underlined that, in the triple network model, glutamatergic excitatory transmission is predominant in the connections within and across networks.

5.5 Current Treatments for Posttraumatic Stress Disorder

Psychotherapy is generally considered first-line treatment for PTSD. A recent meta-analysis showed that effect sizes for trauma-focused psychotherapies versus active control conditions are greater than medications versus placebo (Lee et al. 2016). Strikingly, PTSD is one of the neuropsychiatric disorders with the shortest list of drugs approved for treatment by regulatory agencies. Only the selective serotonin reuptake inhibitors (SSRI) antidepressants paroxetine and sertraline have been approved by the US Food and Drug Administration (FDA) for PTSD treatment. There are many reasons for this, including the complex and highly variable symptom profile (based on subjective patient report), the frequent comorbidity with other neuropsychiatric disorders (e.g., depression, anxiety, substance abuse), the well-known difficulty of treating PTSD patients (particularly military veterans), and last but not least a pathophysiology far from being completely understood. Based on the DSM-V manual, there are 636,120 ways for an individual to qualify for a diagnosis of PTSD (Galatzer-Levy and Bryant; 2013; Maeng and Milad 2017). Also, the recent guidelines for treatment of major agencies differ significantly, although in general they agree on a better efficacy of psychotherapy, including trauma-focused, prolonged exposure, cognitive processing and cognitive-behavioral psychotherapy, eye movement desensitization, reprocessing, compared to pharmacotherapy (Bestha et al. 2018). As a result, off-label prescription and polypharmacy have been nearly a routine in PTSD pharmacotherapy.

Indeed, a shortlist of the medications that have been used for PTSD therapy may resemble a handbook for therapy of the main neuropsychiatric disorders and is too long to be reported fully in this chapter. We will mention here the main classes of drugs used over the years and the principal representatives for each class (Steckler and Risbrough 2012; Maeng and Milad 2017; Abdallah et al. 2018; Bestha et al. 2018).

Traditional Antidepressants Besides paroxetine and sertraline, other SSRI are commonly used, such as fluoxetine. SSRI showed efficacy in reducing symptom severity and in relapse prevention. However, about 60% of patients respond to treatment and only 20–30% achieve full remission. The serotonin and noradrenaline reuptake inhibitor (SNRI) venlafaxine has also been used in some studies, with effect size comparable to SSRI. Among other antidepressants used are mirtazapine, trazodone and the first-generation tricyclic drug amitriptyline. Mirtazapine and trazodone have been used particularly for their sedative effect, but did not become first-line treatment for PTSD because they are less well tolerated.

Anticonvulsants and Antipsychotics Anticonvulsants used include gabapentin, topiramate, and valproic acid. Although some studies found their effect in PTSD promising, recent clinical trials did not confirm efficacy. Also atypical antipsychotics, in particular risperidone, olanzapine and quetiapine have been used, often as adjunctive therapy to antidepressants. The most extensively studied is risperidone, which in early studies showed potential for use in monotherapy or as adjunct to antidepressants. However, a large clinical trial with risperidone augmenting SSRI and meta-analyses articles did not find significant effect (Krystal et al. 2011).

Adrenoceptor Antagonists Noradrenergic hyperreactivity linked to hyperarousal symptoms has traditionally been considered an interesting target. Prazosin, an α1-adrenergic receptor antagonist, showed early promise in treatment of PTSD, particularly for nightmares and sleep-related symptoms. However, additional studies and a recent large randomized controlled trial failed to reproduce these effects, both for overall PTSD and sleep-related symptoms (Raskind et al. 2018). Interestingly, in the randomized control trial in which prazosin was found to be effective, the patients had higher blood pressure than in the later study. This may suggest that higher standing blood pressure could be a biomarker to identify PTSD patients that may benefit from prazosin. It is noteworthy that prazosin is considered as first-line drug treatment by some agencies (e.g., International Society for Traumatic Stress Studies) but not others (US Veteran Administration). The classical β-adrenoceptor blocker propranolol has been considered a promising candidate drug to be administered immediately after traumatic stress to prevent development of PTSD, but clinical trials found it devoid of efficacy when given after trauma (Amos et al. 2014; Sijbrandij et al. 2015).

Benzodiazepines Benzodiazepines have often been prescribed in PTSD to reduce hyperarousal. However, when systematically tested they failed to show therapeutic effects in PTSD. Rather, they showed worsening of PTSD symptoms or increased risk of developing PTSD in the aftermath of trauma.

Glucocorticoids The interest in using GR agonists for treatment of PTSD mainly stems from the observation that individuals who develop PTSD have been shown to have lower cortisol levels at the time of trauma. The tendency for somewhat reduced cortisol levels appears to be maintained during the life course of PTSD patients and seem to correlate with the risk of PTSD development (Yehuda 2004). Reduced cortisol would result in reduced feedback, required for termination of the stress response and prolonged activation of the HPA axis and sympathetic nervous system, long after trauma exposure. Based on this hypothesis it was suggested that administration of a GR agonist in the vicinity of the traumatic event may block development of PTSD. Indeed, beneficial effects of hydrocortisone (the pharmaceutical term for cortisol) have been reported in small, randomized clinical trials (Schelling et al. 2001; Weis et al. 2006). Hydrocortisone augmentation of exposure psychotherapy in veterans was associated with greater reduction in total PTSD symptoms compared to placebo (Yehuda et al. 2015). In a prevention-oriented approach, it would be interesting to treat subjects in the early aftermath of trauma to see if this reduces the development of PTSD in subjects at risk. This approach has been applied, by treating trauma survivors within 6 h with hydrocortisone (Zohar et al. 2011). Two systematic meta-analyses considered several studies using various pharmacological treatments for prevention of PTSD. While all other drugs were ineffective, hydrocortisone showed a large effect in reducing the risk of subsequent PTSD (Amos et al. 2014; Sijbrandij et al. 2015). GR antagonists have also been investigated in preclinical studies but their efficacy is at best uncertain in possible clinical applications (Golier et al. 2016).

5.6 New and Emerging Targets for Treatment of Posttraumatic Stress Disorder

In recent years, many compounds have raised interest as candidate drugs for treatment of PTSD (Steckler and Risbrough 2012; Krystal et al. 2017; Abdallah et al. 2018; Bestha et al. 2018; Sartori and Singewald 2019). Most of them are substances that have been used before with different indication or for recreational use, or experimental drugs employed in preclinical studies. Many are currently tested in ongoing clinical trials. The major classes of these drugs are the following: (1) *Glutamatergic agents*, including ketamine, riluzole, D-cycloserine, *N*-acetylcysteine; (2) *Cannabinoids*, including Δ-9-tetrahydrocannabinol (THC), cannabidiol (CBD); (3) *Neuroactive steroids*, including hydrocortisone, ganaxolone; (4) *Recreational drugs*, including MDMA, psilocybin, LSD; (5) *Opioids*, mainly buprenorphine. Indeed, in a recent survey submitted to several PTSD investigators, asking them to rank the top five potential new therapeutic targets for PTSD, the resulting mechanisms (from 1 to 5) were: (1) NMDA receptor antagonists, (2) cannabinoid receptor modulators, (3) glucocorticoid receptor agonists, (4) non-5-HT reuptake inhibitor antidepressants, (5) opioid receptor agonists (Krystal et al. 2017).

Not casually, these two lists are largely overlapping. Agents acting on the glutamate system, which are a main focus of this article, are treated in separate sections below.

5.6.1 New Glutamatergic Agents for Treatment of Posttraumatic Stress Disorder: The Ketamine Story

The experimental drug that has paved new avenues for the development of glutamate-based treatment strategies for neuropsychiatric disorders is undoubtedly ketamine. Ketamine is a non-competitive antagonist of the NMDA receptor that has been used as anesthetic since the 1960s, but has also been popular as a recreational drug that induces dissociative behavior and has abuse liability. Several clinical studies in the last two decades, mostly with treatment-resistant depressed subjects, have shown that a single infusion of a subanesthetic dose of ketamine (typically 0.5 mg/kg) exerts a rapid (within hours) and sustained (at least a week or longer) antidepressant effect (Berman et al. 2000; Zarate et al. 2006; aan het Rot et al. 2010; Murrough et al. 2013). This has been called by many the most important discovery in half a century in psychopharmacology of mood and anxiety disorders (Duman and Aghajanian 2012). The efficacy of ketamine, confirmed over time by many independent studies, has shown that a drug that targets directly the glutamate system may have a faster antidepressant action, compared with traditional antidepressants targeting monoaminergic systems. Indeed, the story of ketamine is a further demonstration of the neuroplasticity/glutamatergic hypothesis of depression (Sanacora et al. 2012; Duman and Aghajanian 2012; Popoli et al. 2012; Musazzi et al. 2013; Duman et al. 2016; Lener et al. 2017; Murrough et al. 2017). In 2019, after three short-term clinical trials and one longer-term maintenance-of-effect trial, FDA granted approval for the use of esketamine (the S-ketamine enantiomer) nasal spray in conjunction with an oral antidepressant, for treatment-resistant depression. Because of the risk of serious adverse outcomes resulting from sedation and dissociation, and the potential for abuse of the drug, esketamine is only available through a restricted distribution system and administered in a certified doctor's office or clinic.

As mentioned in the previous section, ketamine and other glutamatergic compounds are considered a top choice among experimental drugs for PTSD. A first randomized clinical trial has tested the efficacy of ketamine in chronic PTSD patients against midazolam, used as active comparator. The effect of ketamine infusion, assessed after 24 h, was a significant and rapid reduction in PTSD symptom severity, compared with midazolam. Ketamine was also associated with reduction in comorbid depressive symptoms and improvement in overall clinical presentation. This study provided the first evidence for rapid reduction in symptom severity following ketamine infusion in patients with chronic PTSD (Feder et al. 2014).

Several additional clinical trials with ketamine are reported in the database at clinicaltrials.gov (spring 2020), including single or repeated administration for

therapy of PTSD, on antidepressant-resistant PTSD, on PTSD with wound-related pain (ketamine + opiate), on the possible restoration by ketamine of synaptic loss (measured by detection of SV2A marker of synaptic vesicles; MRI/PET study) (Holmes et al. 2019). Many of these studies are ongoing but some are nearly completed and should contribute to the available evidence on ketamine as a therapeutic drug for PTSD.

5.6.2 The Mechanism of Ketamine

In rodent models, the most striking rapid effect of ketamine is the restoration of neuroarchitecture compromised by chronic stress. Traditional antidepressants, such as SSRI, need several weeks of treatment to reverse dendritic atrophy and loss of spines in pyramidal neurons (Norrholm and Ouimet 2001; Bessa et al. 2009). Indeed, a number of studies showed that a single administration of ketamine in just 24 h may rescue dendritic atrophy and loss of synaptic spines induced by chronic stress. This effect is temporally coincident with the peak of ketamine antidepressant action, and seems to be closely correlated with the behavioral antidepressant effect (Li et al. 2011; Tornese et al. 2019a; Moda-Sava et al. 2019; Treccani et al. 2019; Zhang et al. 2019). The mechanism of ketamine has been extensively investigated in recent years, probably more than any traditional antidepressant before, not only in relation to its rapid antidepressant action, but also in an attempt to better understand the basic neurobiological underpinnings of stress-related psychopathology. There are several components to the mechanism of ketamine. The most well-known are the first two mechanisms proposed, which are as follows: (1) Inhibition by ketamine of NMDA receptors of GABAergic neurons in PFC, which rapidly increases the release of glutamate from pyramidal neurons, activating postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. This induces rapid and transient activation of mammalian target of rapamycin complex (mTORC) signaling and, in turn, increases local expression of synaptic proteins, required for restoration of glutamatergic synaptic spines; (2) Inhibition by ketamine of NMDA receptor-mediated spontaneous transmission in hippocampus. This in turn reduces phosphorylation of and activates eukaryotic elongation factor 2, resulting (again) in local expression of synaptic proteins. Both mechanisms are purported to increase local (synaptic) expression of Brain-Derived Neurotrophic Factor (BDNF), a neurotrophin essential for synaptic function and plasticity (Li et al. 2010; Autry et al. 2011). Several additional mechanisms have been proposed, also involving different brain areas (e.g., Lateral Habenula; Yang et al. 2018). For detailed recent reviews on the mechanism of ketamine, see Zanos and Gould (2018) and Kadriu et al. (2019). An attractive general hypothesis, which stems from numerous preclinical lines of evidence, suggests that ketamine repairs disrupted brain connectivity, possibly by stabilizing excitatory (but also inhibitory) transmission within and between brain areas relevant for pathophysiology (Thompson et al. 2015; Duman et al. 2016; Musazzi et al. 2017; Workman et al. 2018; Zanos et al. 2018). In

particular, a net effect of ketamine (and of its metabolite hydroxynorketamine; Zanos et al. 2016) seems to be a sustained activation of AMPAR-mediated excitatory transmission, which results in increased input to subcortical areas, thereby resulting in better control of the stress response. How ketamine may stabilize excitatory transmission is not clear. One of the first known effects of ketamine is a rapid and transient increase of extracellular glutamate in PFC, assessed by early microdialysis studies (Moghaddam et al. 1997), but there is scarce evidence in the literature on the prolonged effect of ketamine treatment on glutamate release.

A recent study using the chronic mild stress (CMS) protocol, perhaps the most popular rodent model of depression, investigated the effect of a single administration of subanesthetic ketamine (10 mg/kg) on depressed-like behavior and related cellular/molecular changes (Tornese et al. 2019a). Different versions of the CMS protocol have been used by several groups to investigate ketamine's rapid antidepressant effects (Li et al. 2011; Ma et al. 2013; Papp et al. 2017); in those studies typically the effect of ketamine was assessed in stressed rats (taken as a whole group), compared with non-stressed controls. Tornese et al. deemed the rats vulnerable or resilient to the effects of CMS by using the classical sucrose consumption test for anhedonia (one of two core symptoms of depression) and treated the vulnerable rats with ketamine 24 h before the end of CMS (5 weeks). They found that anhedonic behavior and most stress-induced maladaptive changes in the hippocampus were observed only in vulnerable rats, including marked reduction of basal and depolarization-evoked glutamate release, impaired trafficking of BDNF mRNA in dendrites and atrophy of dendrites in CA3 and CA1. Administration of subanesthetic ketamine to vulnerable rats rescued anhedonic behavior, basal glutamate release, BDNF mRNA dendritic trafficking, and dendritic atrophy. Therefore, ketamine in just 24 h was able to rescue depressed-like behavior and related cellular/molecular changes, that were the result of 5 weeks of stress. Noteworthy, one of the key readouts that were restored by ketamine was impaired basal release of glutamate; this suggests that ketamine does not just induce a rapid and transient burst of glutamate release, but rather stabilizes a correct level of synaptic glutamate after stress-induced long-term impairment.

However, again, the outcome of chronic stress protocols in rodents has been related to depression but, in PTSD models, a PTSD-like phenotype should rather be the result of acute, short-term exposure to stressors (Siegmund and Wotjak 2006; Musazzi et al. 2017). Therefore, in preclinical studies investigating the possible pharmacological action of ketamine in PTSD, it should be assessed if ketamine may block the long-term pathology-related maladaptive effects of acute stress exposure.

5.6.3 A Prophylactic Action of Ketamine Against the Outcome of Traumatic Stress?

Recent evidence suggested that ketamine may exert a prophylactic action against the effects of stressors, perhaps by enhancing resilience. In one study, mice were

administered a single injection of ketamine (30 mg/kg) and 1 week later were subjected to chronic social defeat stress (SDS), a protocol that induces depressive-like behavior. Ketamine-treated mice were protected against the maladaptive effects of SDS, showing reduced immobility time in the forced swim test; ketamine was ineffective if administered 24 h after conclusion of SDS protocol. A similar prophylactic, protective effect of ketamine was found when it was administered before the Learned Helplessness protocol or chronic CORT administration. It was suggested that ketamine has a prophylactic effect when administered before, but not after, stress (Brachman et al. 2016). Furthermore, the effects of ketamine were tested in a contextual fear conditioning protocol (see Sect. 5.1.1 above), in which the administration of prophylactic ketamine 1 week before, but not after the protocol, reduced freezing behavior, facilitating fear extinction. This confirmed that ketamine should be administered before exposure to stressors (McGowan et al. 2017). In a different study, Amat et al. (2016) found that 10 mg/kg ketamine, administered 2 h, 1 week or 2 weeks before inescapable acute tail shocks, prevented the reduction of social investigation in rats, an effect replicated by microinjection of ketamine into PL region of the mPFC.

The idea that ketamine (or other suitable compounds) may exert a prophylactic action in PTSD is certainly attracting. Subjects at risk could be treated before being exposed to potentially traumatic environmental situations. However, it would certainly be more feasible to treat people after they have been exposed to traumatic shock, such as in the aftermath of a natural catastrophe. The appropriate time window after exposure to traumatic stress should be investigated, in order to optimize the prophylactic effect of treatment against subsequent development of PTSD.

This possibility was investigated, again by measuring glutamate release from superfused PFC synaptosomes (see Sect. 5.3.1 above), obtained by rats subjected to inescapable acute FS stress. Previous work showed that FS stress-induced enhancement of glutamate release in PFC is blocked by prior chronic treatment with traditional antidepressants (Musazzi et al. 2010, 2013). Preliminary results showed that a single ketamine injection (10 mg/kg) blocked the stress-induced enhancement of depolarization-evoked glutamate release if administered either 72 or 24 h, but not 1 or 2 h, before FS. Moreover, ketamine administered 6 h after FS stress completely blocked the enhancement of glutamate release measured 24 h after stress exposure and promoted extinction of contextual fear memory (Popoli 2018; Tornese et al. 2019b).

Therefore, although more work is necessary to understand better modality and timing of treatment, the possible implementation of a prophylactic use of ketamine to prevent the development of PTSD and other stress-related disorders is a promising perspective.

5.6.4 *Other Glutamatergic Agents for Treatment of Posttraumatic Stress Disorder*

Other agents targeting the glutamate system that have been proposed for the treatment of PTSD are D-cycloserine (DCS), riluzole, and *N*-acetylcysteine (NAC). DCS, similar in structure to the amino acid alanine, is a partial agonist of NMDA receptor that was shown to enhance fear extinction learning in several experimental protocols, including the single prolonged stress protocol (Yamamoto et al. 2008; Ledgerwood et al. 2005).

Fear extinction learning requires protein synthesis and long-lasting synaptic plasticity, involving the MAPK/Erk, PI3K/Akt, and BDNF pathways. Activation of NMDA receptor, which stimulates the MAPK/Erk pathway via Ca2+ influx, has been shown to be highly relevant in building extinction memory, making DCS currently the most studied adjunct treatment to exposure therapy in off-label use for anxiety-related disorders (Sartori and Singewald 2019). A recent meta-analysis examined several clinical studies testing DCS as adjunct to exposure therapy for anxiety, obsessive-compulsive disorder (OCD), and PTSD. The authors found evidence supporting the short-term superiority of DCS vs placebo in the augmentation of exposure therapy and mixed evidence supporting maintenance of the benefits at follow-up. Although statistically significant, the effect sizes were small. Additional ongoing studies are currently evaluating the use of DCS in combination with psychotherapy. However, data on the efficacy of DCS in the modulation of associative fear learning and treatment of PTSD are mixed. In healthy volunteers, DCS facilitated consolidation of fear acquisition and cued fear extinction, while other studies did not find a reduction in conditioned fear. DCS was found particularly effective when administered with virtual reality exposure therapy, and in subjects with more severe PTSD. Moreover, participants with high conscientiousness and low extraversion exhibited better outcomes with DCS and exposure therapy. These data may suggest that DCS is an effective adjunct therapy only for a subset of the clinical population and with specifically tailored psychotherapy sessions (Bowers and Ressler 2015).

Riluzole is a neuroprotective agent, approved by FDA for treatment of Amyotrophic Lateral Sclerosis, which targets primarily the glutamate system and has a complex mechanism of action. It blocks voltage-sensitive sodium channels with high affinity for inactivated channels, resulting in the inhibition of high-frequency neuronal firing. At higher concentrations, riluzole interacts with other presynaptic voltage-gated ion channels, including calcium and potassium channels. The main mechanism proposed is a reduction of glutamate release and excitatory transmission, together with increase of glutamate clearance by transporters and of synaptic AMPA receptor trafficking. Riluzole has been shown to reduce clinical symptoms in OCD, Generalized Anxiety Disorder and major depression with anxiety comorbidity (Pittenger et al. 2008). In 2018, positive results were reported from a proof of concept clinical trial investigating the acute effect of a single sublingual dose of riluzole (BHV-0223; under license of Biohaven) in patients with Social

Anxiety Disorder, after meeting the primary endpoint of the trial. Two clinical trials for PTSD are reported on ClinicalTrials.gov, an open-label and a randomized trial currently evaluating safety and efficacy of oral riluzole either as monotherapy or as augmentation treatment in patients with PTSD.

The antioxidant NAC has been increasingly investigated as a therapeutic agent for a variety of neuropsychiatric disorders, characterized by impairment of executive functions, impulse control, and top-down regulation, including Substance Use Disorder (SUD). It has been shown that in cocaine relapse NAC stabilizes extracellular glutamate by restoring the activity of cystine/glutamate exchanger in the nucleus accumbens (Baker et al. 2003). Moreover, animal models of addiction showed chronic downregulation of the glial glutamate transporter in the nucleus accumbens, and treatment with NAC restored the transporter, thereby normalizing glutamate transmission (Kalivas and Volkow 2011). Co-occurrence of PTSD with SUD is not unusual and it has been suggested there are neurobiological mechanisms in common, such as impaired prefrontal cortex regulation of basal ganglia circuitry, particularly for glutamate synapses in the nucleus accumbens. Based on the capacity of NAC to stabilize glutamate synapses in nucleus accumbens and to inhibit drug use in animal models and human addiction, NAC was tested as adjunct therapy for cognitive-behavioral therapy in a placebo-controlled trial with comorbid PTSD/SUD. The results provided encouraging preliminary support for combining NAC and cognitive-behavioral therapy in patients with PTSD and SUD (Back et al. 2016). Two additional clinical trials are reported in the ClinicalTrials.gov database, one withdrawn and another one recruiting patients.

5.6.5 New Non-Glutamatergic Agents for Treatment of PTSD

Although some of the agents reported in this section are not entirely new to the PTSD field, we briefly discuss here the remaining 4 classes that were considered the top potential new therapeutic targets for PTSD in a recent survey (Krystal et al. 2017).

Cannabinoids The endocannabinoid (eCB) system plays a key role in the modulation of fear, anxiety, and stress response. Most eCB effects in brain are mediated by the CB1 receptor, which is present on presynaptic terminals of most major neurotransmitter systems (including glutamate and GABA), although CB2 and TRP1 receptors also play a role. Converging evidence showed that the eCB system is dysregulated in stress animal models and in patients with PTSD. Chronic stress resulted in lower eCB concentrations and upregulation of CB1 receptors; subjects with PTSD or major depression showed reduced circulating levels of the eCB anandamide and upregulation of CB1 receptors in several areas (Morena et al. 2016; Hill et al. 2018). Activation of eCB signaling has been shown to exert anxiolytic and antidepressant-like effects in rodents. Several clinical studies suggested that enhancement of eCB signaling may be a promising target to reduce

PTSD symptoms (Sbarski and Akirav 2020). With regard to PTSD, the eCB agonists that have been investigated in preclinical studies are THC and CBD. THC has been shown to have biphasic effects, where low doses are anxiolytic and high doses are anxiogenic; instead, CBD lacks the psychoactive effects of THC and seems to have restorative properties in various pathophysiological situations (Crippa et al. 2018). CBD may facilitate the extinction of contextual fear memory and decrease the salience of significant stimuli (Bitencourt et al. 2008). In clinical studies THC administration for 3 weeks as add-on resulted in improvement of global symptoms of PTSD, with mild adverse effects in some patients. CBD treatment for 8 weeks resulted in decrease of PTSD symptom severity. Nabilone, a synthetic derivative of THC, was also employed in three studies, with general reduction in severity of symptoms (Sbarski and Akirav 2020, and refs. therein). Currently, reported on the ClinicalTrials.gov website and related to PTSD with different modalities, there are 7 studies with THC or cannabis, 3 studies with CBD (PTSD with or without alcohol use disorder) and 2 with nabilone (in PTSD with cannabis use or as add-on to THC treatment).

GR Agonists As addressed above in Sect. 5.5, glucocorticoids have been an option for treatment of PTSD for quite some time and, at least for preventive treatment, have often rated superior to other drugs available. A thorough discussion of their effects and rationale for use in PTSD is beyond the finality of this chapter. For a review see de Quervain et al. (2019). Currently, there are several ongoing clinical trials investigating the effect of hydrocortisone on the development of PTSD and for established PTSD.

Non-5-HT Reuptake Inhibitor Antidepressants As addressed above in Sect. 5.5, besides SSRI, several other antidepressants with different mechanisms have been employed over the years, including the dual inhibitor venlafaxine, the tricyclic antidepressants imipramine, desipramine, the MAO inhibitor phenelzine, the mixed-activity drugs mirtazapine, trazodone, nefazodone, tianeptine, vortioxetine. Most of them were investigated in small-sample clinical trials. While some of them seemed nearly as effective as SSRI, none of them became first-line treatment, partly also because they are less well tolerated. In a network meta-analysis, encompassing several classes of drugs (including antipsychotics, mood stabilizers, anticonvulsants, and other mechanisms), many of these drugs were evaluated. Desipramine, phenelzine, venlafaxine were more effective than placebo; phenelzine was significantly more effective than nearly half of all active treatments assessed and was the only drug that was significantly better than placebo for number of dropouts (Cipriani et al. 2018). Currently, ongoing clinical trials for venlafaxine, desipramine, trazodone, mirtazapine and vortioxetine are reported on the ClinicalTrials.gov website.

Opioid Receptor Agonists Early studies have suggested that morphine administration shortly after traumatic stressors reduces the risk of developing PTSD (Holbrook et al. 2010). Moreover, opioid-use disorder often occurs in comorbidity with PTSD because patients will self-treat with opiates in an attempt to relieve the psychic suffering from PTSD. Buprenorphine, a partial agonist of μ opioid receptors and

antagonist of κ opioid receptors has been tested for treatment. Buprenorphine was administered in combination with naloxone, compared to either SSRI or other opioids (Lake et al. 2019). Buprenorphine/naloxone showed a statistically significant improvement in PTSD scores as compared to SSRI. Currently, there are 8 ongoing clinical trials testing buprenorphine in PTSD, mostly in patients with comorbid opioid-use disorder.

5.6.6 *3,4,-methylenedioxmethamphetamine (MDMA) for Treatment of Posttraumatic Stress Disorder*

MDMA, commonly known as ecstasy or molly, is a psychoactive drug primarily used for recreational purposes. MDMA is a ring-substituted phenethylamine, which acts primarily by increasing the activity of the neurotransmitters serotonin, dopamine, and noradrenaline and also elevates the levels of the hormone oxytocin. MDMA is illegal in most countries and has had so far no approved medical uses. In 2017 FDA designated MDMA-assisted psychotherapy for PTSD a Breakthrough Therapy, with agreement on Special Protocol Assessment for phase 3 Trials, based on data showing a large effect size for this treatment (Sessa 2017; Feduccia et al. 2019). The Phase 3 trials are expected to be completed in 2021, meaning that the FDA could approve the treatment as early as 2022. The European Medicines Agency also approved the study to move to phase 3. A comparison of data used for the approval of paroxetine and sertraline (the two SSRI currently approved for PTSD therapy) and pooled data from Phase 2 studies showed that MDMA-assisted psychotherapy was a substantial improvement over available pharmacotherapies in terms of safety and efficacy, with lower dropout rates compared to sertraline and paroxetine (Feduccia et al. 2019). It is speculated that MDMA augmentation of psychotherapy action involves enhanced fear extinction, memory reconsolidation, enhanced therapeutic alliance, widening of a window of tolerance for distressing thoughts or experiences, and re-opening of a critical period for experiencing social reward. Currently, there are 17 ongoing clinical trials testing MDMA-assisted psychotherapy for treatment of PTSD. The MDMA-PTSD chapter is part of the revival in the study of psychedelic drugs potential for therapy of neuropsychiatric disorders, which also includes psilocybin in anxiety, depression, smoking, alcoholism, and of course ketamine in treatment-resistant depression (see above) (Nutt 2019).

5.7 Conclusions and Future Perspectives

Although several possible therapeutic options are offered for PTSD, the number of drugs approved for therapy is still quite small. Despite the relative efficacy of SSRI, less than 60% of patients respond to these drugs and only 20–30% achieve full remission.

Recent translational and clinical research has revealed the key role played by glutamate and the glutamatergic system in pathophysiology of neuropsychiatric disorders, including PTSD. As a result, ketamine has become in the last few years the single most investigated drug for therapy of mood and anxiety disorders; this has brought about several new lines of discovery and development of the class of rapid-acting antidepressants. As illustrated in this chapter, ketamine and several other drugs targeting directly NMDA receptors or other mechanisms in the glutamate system showed an interesting potential for therapeutic effect. In particular, in preclinical studies, converging evidence showed that subanesthetic ketamine may exert a prophylactic action against the development of PTSD-like behavior, possibly when administered shortly after traumatic stress. Controlled clinical trials should check this action of ketamine in trauma survivors, also to identify the right time window for intervention. Moreover, current and future clinical trials with additional glutamatergic drugs will show if any of these other drugs should be employed for therapy of this serious, debilitating mental illness.

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Chapter 6
Glutamate in Migraine Neurobiology and Treatment

Anna P. Andreou

Abstract Migraine is a disabling chronic condition characterised by recurrent episodes of head pain accompanied by other sensory disturbances. Its pathophysiology is complex and involves both the peripheral and central nervous systems. Glutamate is believed to play an important role in migraine pathophysiology, as it is involved in multiple processes of migraine's neurobiology. Glutamate is the main neurotransmitter of the trigeminal system and along the ascending trigeminothalamic pathways. It is also involved in the initiation and progression of cortical spreading depression, the underlying biological processes of migraine aura. Its levels are increased during attacks and in chronic migraine patients. Increased glutamate excitation is believed to be at least partly responsible for the clinical symptoms of allodynia in patients during an attack, as well as in the transformation of episodic migraine to chronic migraine. Some of the current migraine treatments include in their mechanism of action, at least partly, modulation of glutamatergic signalling. While some attempts have been made to directly block glutamate receptors, these were abandoned due to the development of significant side effects. Future glutamatergic therapeutics that could indirectly block glutamatergic signalling may present a viable effective tool in migraine patients.

Keywords Glutamate · Migraine · Aura · Spreading depression · Trigeminal · CGRP · Central sensitization

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_6

6.1 Introduction

Migraine is a common, chronic neurological disease characterised by recurrent episodes of intense headache, that can worsen with activity, accompanied by nausea, sensitivity to light, noise or even smells (IHS 2018). A proportion of patients also experiences migraine with aura—transient neurological symptoms that usually occur just before the onset of a migraine headache. About 1–2% of migraine patients develop chronic migraine, characterised by at least 15 headache days per month (Buse et al. 2012). The majority of these patients may actually develop a daily or nearly daily migraine headache. Migraine pathophysiology is now believed to be triggered by, or at least include, dysfunctional processes at the level of the hypothalamus (Peres et al. 2001; Schulte et al. 2017; Schulte and May 2016), making migraine a brain disorder. However, many of the successful treatments of migraine involve drugs that do not cross the blood brain barrier, with their mechanism of action identified at the peripheral nervous system, mainly the trigeminal nerve and the trigeminal ganglion (Andreou and Edvinsson 2019; Lambru et al. 2018).

The pain of migraine was initially thought to be driven by the cephalic vasculature, potentially through excess vasodilation (Moskowitz and Macfarlane 1993; Wolff 1948). More recent research in migraine pathophysiology suggests that dysfunctional brain networks are involved in the disorder's predisposition and potentially in driving attack initiation (Andreou and Edvinsson 2019). However, central and peripheral neuronal pathways involved in pain signalling, as well as inflammation, are equally important drivers of disease biology and offer targets for the development of future therapeutics. Glutamate is the excitatory neurotransmitter that drives activation of the both the peripheral and central arms of the trigeminal pain pathway, making it a key player in the manifestation of migraine. Migraine pain-relay centres, including the trigeminal ganglion, trigeminocervical complex (TCC) and sensory thalamus, contain glutamate-positive neurons (Alam et al. 1998; van Dongen et al. 2017), while glutamate has been shown to excite neurons in the TCC and thalamus (Ferrari et al. 1990; Martinez et al. 1993). The presence of glutamate in the transmission of sensory information implicates the involvement of glutamate receptors that modulate glutamate responses, in migraine neurobiology. Thus, glutamate receptors and glutamatergic signalling offer a great potential in the development of novel, migraine-specific treatments.

In the present chapter we will discuss the neurobiology and pathophysiology of migraine and the current evidence on the involvement of glutamate signalling in the development of different migraine symptoms. We will further discuss interactions with the glutamatergic signalling of current migraine treatments and how we could effectively target the glutamate system in the future in order to develop more effective, tolerable, migraine-specific treatments.

6.2 Plasma, Cerebrospinal Fluid (CSF) and Brain Levels of Glutamate in Migraine Patients

Migraine patients have interictal elevated plasma levels of neuronal amino acids, including glutamate, glutamine, glycine, cysteic acid and homocysteic acid (van Dongen et al. 2017). The plasma levels of glutamate were found to be further increased during a migraine attack (Alam et al. 1998; Ferrari et al. 1990). Increased peripheral glutamate, if correlated with increased brain levels, suggests that migraine biology involves a persistent neuronal hyperexcitability that becomes heightened during a migraine attack.

Indeed, during migraine attacks cerebrospinal fluid (CSF) concentrations of glutamate were found to be higher in patients than in controls, suggesting an excess of neuroexcitatory amino acids in the CNS (Martinez et al. 1993). The increased levels of glutamate, particularly in migraine with aura patients may be relevant to their neurological symptoms (D'Andrea et al. 1991). Additionally, glutamate concentrations were increased in CSF from chronic migraine patients (Gallai et al. 2003; Peres et al. 2004; van Dongen et al. 2017), further supporting the concept of excess neuroexcitation in the CNS. In support of this theory is the finding that migraine patients exhibit signs of central sensitization during an attack (Burstein et al. 2000), which is a process of excessive activation of second order dorsal horn neurons and/or third order thalamic neurons, which occurs following peripheral sensitization in the trigeminal ganglion (Woolf and Decosterd 1999). Glutamate release in the spinal dorsal horn and glutamate receptor activation mediates central sensitization (Burstein 2001). Allodynia and hypersensitivity is a common clinical observation of chronic migraine (Bigal et al. 2008a; Kitaj and Klink 2005) and is regarded as the result of central sensitization (Andreou and Edvinsson 2019).

Brains of patients with migraine differ pharmacologically from those of non-migraine sufferers (Mathew 2011), with glutamate playing a major role in such differences. A small magnetic resonance spectroscopy study found glutamatergic abnormalities in the anterior cingulate cortex and insula in migraine patients during their interictal period compared to healthy controls (Prescot et al. 2009). Bigger magnetic resonance spectroscopy studies of the cortex and the thalamus found higher interictal glutamate levels in the visual cortex and thalamus of migraine patients (Bathel et al. 2018; Zielman et al. 2017), but no group differences in GABA levels supporting the hypothesis of cortical and thalamic hyperexcitability in migraine driven by excess availability of glutamate (Bathel et al. 2018).

6.3 Genetics of Migraine and the Glutamatergic System

The increased glutamate levels, both during a migraine attack and in chronic migraine, may suggest a defective cellular reuptake mechanism for glutamate in migraine patients at the neuronal/glial level, predisposing the pain pathway to excess excitation. Increased cortical glutamate levels may also drive the development of cortical spreading depression during migraine aura. To date, our knowledge on migraine genetics is not complete, but some genetic modification findings may support this theory.

Migraine is a multifactorial disorder and genetic factors play an important role in the development of the disorder. Studies examining the genetic basis of migraine are complicated by the heterogeneous nature of the condition and the lack of objective clinical or diagnostic tests. Family and twin studies showed increased risk in the family members of migraine patients (Noble-Topham et al. 2003; Ziegler et al. 1998), indicating that genetic factors are a major contribution to the pathogenesis of both migraine with and without aura. Genome-wide association studies (GWAS) failed to shed light on the actual molecular changes that are responsible for the genetic susceptibility of migraine. It is understood that multigenetic variants, rather than individual genes, influence the susceptibility to migraine (Gormley et al. 2016). Regardless of these outcomes, due to their small effect size, no single nucleotide polymorphisms has any clinical use in predicting the risk of developing migraine (Andreou and Edvinsson 2019). Potentially, more knowledge on the function of these variants could highlight which molecular pathways are involved in migraine susceptibility (van den Maagdenberg et al. 2019). With respect to the glutamatergic system, one of the chromosomal regions with significant linkage for non-hemiplegic migraine with aura is the 11q24 locus (Cader et al. 2003), which maps, among other candidates, the GRIK4 gene of the KA1 kainate receptor subunit (Mayer 2007). Polymorphisms in the glutamate receptor ionotropic amino-3-hydroxy-5-methyl-4-isoxazole-propionin acid 1 (GRIA1) and GRIA3 genes that code for two of the four subunits of the AMPA ionotropic glutamate receptor have been previously associated with migraine in an Italian and Australian population (Fang et al. 2015; Formicola et al. 2010; Maher et al. 2013), further supporting the plethora of evidence suggesting that glutamate dysfunction may contribute to migraine susceptibility. Furthermore, genetic screening of a patient with hemiplegic migraine, seizures and episodic ataxia revealed a mutation on the excitatory amino acid transporter 1 (EAAT1) (Jen et al. 2005), which reduces the glial cell's ability to clear glutamate from the synaptic cleft (Ramadan and Buchanan 2006). The resultant increased availability of synaptic glutamate would contribute to post-synaptic hyperexcitation, which would further lead to the development of central sensitization and the prominent neurological symptoms seen during migraine attacks. Another small study demonstrated that polymorphism of the glutamate transporter protein excitatory amino acid transporter 2 (EAAT2) are potentially involved in the development of medication-overuse headache and migraine transformation into chronic daily

headache, as *EEAT2* polymorphisms were significantly higher in patients with frequent analgesic usage (Shin et al. 2011).

Familial Hemiplegic Migraine (FHM) is a rare monogenic form of migraine with prominent aura symptoms (Ferrari et al. 2015) that is inherited in an autosomal dominant manner. The molecular linkage of FHM involves three mutations (van den Maagdenberg et al. 2007): FHM 1 mutation affecting the *CACNA1A* calcium channel gene mapped to chromosome 19p13 (Ophoff et al. 1996). FHM 2 mutation affecting the *ATP1A2* gene on chromosome 1q23 (De Fusco et al. 2003). FHM 3 mutation affecting the *SCN1A* gene on chromosome 2q24, which is a rarer cause of FHM (Dichgans et al. 2005; Vanmolkot et al. 2007). Interestingly a common consequence of these mutations is an increase in glutamate availability at the synaptic cleft (Andreou and Goadsby 2009a). The FHM 1 mutation on the pore-forming A1 subunit of $Ca_v2.1$ (P/Q-type) voltage-gated neuronal calcium channels that modulate release of neurotransmitters at peripheral and central synapses (van den Maagdenberg et al. 2007; Wessman et al. 2004) can have as a consequence enhanced glutamate release due to enhanced calcium flux at the pre-synaptic terminal (Schneggenburger and Neher 2005). The FHM2 mutation affecting the A2 subunit of sodium-potassium pump ATPases, which transport potassium and sodium ions across the cell membrane, has as a consequence a dysfunction on the reuptake of potassium and glutamate from the synaptic cleft into glial cells (De Vries et al. 2006). The FHM 3 mutation affects the A1 subunit of neuronal voltage-gated sodium ($Na_v1.1$) channels that normally modulate generation and propagation of action potentials, and has as a consequence the facilitation of high-frequency discharges that might also increase synaptic glutamate levels (Dichgans et al. 2005). The increased glutamate availability at the synaptic cleft caused by these mutations could potentially explain the increased susceptibility to cortical spreading depression, the underlying mechanism of migraine aura (van den Maagdenberg et al. 2004; Wessman et al. 2007).

6.4 Pathophysiology of the Migraine Attack and Glutamate Involvement

To date, through brain imaging studies we have a clear understanding of the bulk brain structures involved in migraine pathophysiology, however, the exact molecular mechanisms are not understood. Glutamate, as the main excitatory neurotransmitter in the brain, and the major neurotransmitter of the peripheral trigeminal system, has been implicated in all phases of a migraine attack. A migraine attack is characterised by different phases, the premonitory phase, migraine aura phase, the headache phase and the postdrome. Each phase is thought to involve functional changes in different brain structures.

6.4.1 The Premonitory Phase

Before the onset of any neurological symptoms or head pain, the majority of migraine patients can recognise the onset of the "premonitory phase". During the premonitory phase, which can last between few hours to days, patients experience excessive yawning, thirst, somnolence, food craving, cognitive difficulties and mood changes (Laurell et al. 2016). Early hypothesis on the brain areas involved in the preliminary phase of migraine suggested an association with hypothalamic function, given that the symptoms described are strongly associated with homeostatic functions regulated by the hypothalamus, such as arousal, sleep and feeding (Alstadhaug 2009). Additionally, a disturbance in homeostatic function, such as changes in sleep or eating patterns is a significant trigger of attacks (Kelman 2007). In the past years, few brain imaging studies provided stronger evidence for hypothalamic activation in migraine patients. These studies demonstrated increased blood flow in the posterior region of the hypothalamus during the very early stages of spontaneous migraine attacks (Denuelle et al. 2007; Schulte and May 2016) and during the premonitory phase of nitroglycerin (nitric oxide-NO donor)-induced migraine attacks (Maniyar et al. 2014). One of the fMRI studies, which scanned daily a migraine patient and captured all phases of migraine within a period of one month, reported, in addition to hypothalamic activation, increased activity at the occipital cortex (Schulte and May 2016), which has been long recognised as an area of hyperexcitability both in episodic and in chronic migraine (Aurora et al. 1999; Mulleners et al. 2001). Although it has been suggested that dysrhythmia along the thalamo-cortical axis in migraine patients may be responsible for abnormal cortical responses (Coppola et al. 2007), no such theory or evidence has been shown to date for hypothalamic-cortical dysrhythmia. Hence, through which networks, neurotransmitters and molecular changes the occipital cortex and the hypothalamus may influence each other remains unknown. A role for glutamate is possible, as preclinical studies demonstrated the participation of glutamatergic efferent pathways from the cortex to the posterior hypothalamus in the modulation of pain and anxiety and highlighted a role for ionotropic glutamate receptors (Falconi-Sobrinho et al. 2017).

Importantly, to date we do not understand the actual hypothalamic nuclei and their pharmacology involved in the development of the premonitory phase of migraine and potentially the triggering of a migraine attack. The hypothalamus, although a small region in the brain, consists of a number of different subnuclei that play a crucial role in many important functions, including releasing hormones, regulating body temperature, sleep and arousal. Although imaging studies suggest it is mostly its posterior area that could be implicated in the premonitory phase of migraine, several subnuclei, neurotransmitters and neuropeptides may be involved. Mainly animal studies suggest that these include dopaminergic mechanisms (Akerman and Goadsby 2007; Barbanti et al. 1998; Charbit et al. 2010; Marmura 2012; Shepherd et al. 2002), potentially from the dopaminergic A11 nucleus of the hypothalamus which has been shown to project to the TCC (Bjorklund and Skagerberg 1979). Interestingly, vesicular glutamate transporter 2 (VGluT2)

mRNA-expressing neurons are observed within different hypothalamic nuclei and on each midbrain dopamine system, suggesting that at least a subset of neurons might release dopamine and glutamate separately from different varicosities in many of their single axons (Kawano et al. 2006; Morales and Root 2014). To date, no concrete data in humans suggest the actual involvement of any specific hypothalamic nucleus or hypothalamic neurotransmitters/neuropeptides in migraine pathophysiology, although it remains an interesting area to explore for the development of future migraine therapeutics.

6.4.2 The Migraine Aura

The occipital cortex has been strongly linked to the development of migraine aura. This phase of a migraine attack occurs in about 20% of patients (Rasmussen and Olesen 1992) and it is characterised by transient neurological symptoms, most commonly visual alterations, that occur just before, or at the onset the actual migraine headache (IHS 2018; Zhang et al. 2016). Visual symptoms are the most common and are usually described as zigzag or scintillating figures mostly affecting one hemifield of both eyes. In some patients, sensory symptoms affecting the hand and gradually spreading to the whole arm and the perioral region occur alone or in conjunction with visual aura (Russell and Olesen 1996). Motor aura is less frequent and not well recognised by patients and is usually described as motor weakness (Jensen et al. 1986; Silberstein et al. 2001). Speech disturbances may also occur in some patients during the aura phase.

The underlying phenomenon that drives the migraine aura is now believed to be a wave of cortical spreading depression (CSD) which spreads out from the cortex, resulting in an initial hyperaemic phase followed by an oligaemic phase, and linked with a wave of cortical neuronal depolarisation (Lauritzen 1994; Leão 1944; Olesen 1998; Olesen et al. 1990). CSD, described first by Leão in the rabbit cortex in 1944, is a self-propagating depolarisation of neurons and glia linked with depressed neuronal electrical activity (Leão 1944) that moves at a rate of about 2–3 mm/min across the cerebral cortex. Leão first observed that CSD leads to transient dilatation of pial arteries. Following this transient hyperperfusion, hypoperfusion ensues, which persists long after CSD waves have passed. Spreading depression has been demonstrated in almost all the grey matter regions of the central nervous system (CNS) (Somjen 2001), although the cortex of primates, especially in humans, is relatively more resistant to CSD. Early observations from Lashley (Lashley 1941) suggested an association between CSD and the migraine aura and several imaging and blood flow studies of patients during migraine with aura showed unilateral regions of occipital hypoperfusion that tend to spread rostrally from the occipital cortex and persist into the headache phase (Sanchez-del-Rio and Reuter 2004). Actual clinical evidence supporting that a cerebral blood flow altering event such as CSD generates the aura in human visual cortex came only with the use of high-field functional MRI with near-continuous recording during migraine visual aura in

humans. With this method Hadjikhani and colleagues (2001) observed blood oxygenation level-dependent (BOLD) signal changes that demonstrated characteristics of CSD as time-locked to percept onset of the aura. For ethical reasons, direct electrophysiological recordings in the migraine brain have not been contacted, as for example in traumatic brain injury (Hartings et al. 2011; Lauritzen et al. 2011).

It is not yet clear how CSD is triggered in human cortex during migraine aura. A number of diverse stimuli trigger CSD in animal models, including direct cortical trauma, exposure to high concentrations of excitatory amino acids, including glutamate, or K^+, direct electrical stimulation, inhibition of Na^+/K^+-ATPase and energy failure (Somjen 2001). CSD in the neocortex of a variety of species, including man, has been demonstrated to be dependent on activation of the N-methyl-D-aspartate (NMDA) receptor (Faria and Mody 2004). Local release of glutamate by neurons is thought to initiate CSD and the subsequent activation of post-synaptic central glutamate receptors is argued to explain its propagation (Vinogradova 2018; Zandt et al. 2013). Volume-sensitive organic anion channels (VSOACs) in astrocytes are activated by cell swelling and release glutamate which contributed further to the propagation of spreading depression (Basarsky et al. 1999). NMDA receptor antagonists reduce the rate of propagation of SD (Basarsky et al. 1999). Furthermore, inhibition of CSD by memantine, an NMDA receptor antagonist, also suggests a key role for activation of neuronal glutamate receptors in the initiation of CSD (Peeters et al. 2007). As previously mentioned genetic predispositions and environmental factors may modulate individual susceptibility by lowering the CSD threshold (van den Maagdenberg et al. 2004), and cortical excitation may cause sufficient elevation in extracellular K^+ and glutamate to initiate CSD (De Fusco et al. 2003).

Although no obvious aura symptoms are reported by the majority of migraine patients, the presence of silent auras has been proposed (Dahlem and Isele 2013; Purdy 2008), based on observations of increased cortical blood flow in migraine without aura patients at the onset of a migraine attack (Denuelle et al. 2008; Woods et al. 1994). This theory however remains a matter of debate, as it is yet unclear if CSD can trigger a migraine attack in humans. Clinically, this hypothesis is not supported as aura without headache is not uncommon, and migraine aura is not always contralateral to the headache (Goadsby 2001). On the other hand, increased cortical excitability, potentially due to elevated glutamatergic activity, has been seen in migraine without aura patients controls (Aurora et al. 1999; Mulleners et al. 2001). In animals, CSD has been shown to induce activation of second order neurons in the TCC, and the authors suggested this is due to sensitization of pial emended trigeminal fibres from ions released from the cortex during a CSD (Zhang et al. 2010, 2011). However, if indeed the ascending trigeminothalamic pathway can be modulated by CSD, this could also be through activation of cortico-spinal projections or cortico-thalamic activation (Andreou et al. 2012, 2013), at least in animal models of migraine.

6.4.3 The Headache Phase

The headache phase of migraine is the most disabling phase of the attack. The actual pain in an untreated migraine attack may last between 4 and 72 h and it is characterised as moderate or severe. Head pain is often accompanied by nausea and other sensory symptoms, including photophobia and phonophobia. The pathophysiology of the headache phase in migraine is believed to include activation of trigeminal fibres, that innervate the dura matter and intracranial vasculature (Edvinsson et al. 2020; Olesen et al. 2009; Penfield and McNaughton 1940; Ray and Wolff 1940; Wolff 1948). These primary fibres have their cell body in the trigeminal ganglion and project centrally in the trigeminocervical complex (TCC; trigeminal nucleus caudalis, C1 and C2 spinal levels) (Edvinsson et al. 2020). The axons of the second order neurons in the TCC are part of the ascending trigeminothalamic pathway which projects and transmits nociceptive information to third order neurons, mainly in the ventroposteromedial thalamic nucleus (VPM) (Andreou and Edvinsson 2019).

A number of evidence over the decades suggest that activation of peripheral trigeminal fibres and subsequently of the ascending trigeminothalamic pathway during the headache phase may drive the nociceptive signals of the migraine headache (Andreou and Edvinsson 2019). First, stimulation of the dura matter and its vasculature in humans during awake brain surgery induces head pain that resembles the migraine headache and its frequent localisation on the temporal region (Olesen et al. 2009; Penfield and McNaughton 1940; Ray and Wolff 1940; Wolff 1948). Activation of the trigeminal fibres in migraine is mostly evident by the release of the neuropeptide calcitonin-gene related peptide (CGRP). CGRP levels have been shown to be elevated in cranial circulation during a migraine attack and in chronic migraine patients. Animal studies suggest that the origin of the CGRP is indeed the trigeminal nerve (Goadsby et al. 1988, 1990; Lambert et al. 1988). Additionally, substances like calcitonin gene-related peptide and histamine, that do not cross the blood brain barrier (BBB), can trigger a migraine attack (Hansen et al. 2010; Lassen et al. 1995). Final evidence and perhaps the most important is that therapeutics, like triptans (5HT1B/D agonists),—the migraine-specific acute treatments, that do not cross the BBB can stop a migraine attack (Millson et al. 2000; Tfelt-Hansen 2010). Other preventive migraine treatments that also do not cross the BBB, like the new CGRP monoclonal antibodies (mAbs) and botulinum toxin A (BOTOX) are amongst the most effective treatments in reducing the frequency of headache in chronic migraine patients (Andreou et al. 2018; Lambru et al. 2018).

The trigeminothalamic pathway includes the second order neurons located in the TCC and their projections to third order neurons, mainly in the VPM nucleus. Both the TCC and the thalamus are important relay centres of the migraine pathophysiology and prominent sites of action of migraine therapeutics (Andreou et al. 2010; Andreou and Goadsby 2009a; Andreou and Goadsby 2011; Shields and Goadsby 2006). The thalamic area has been further implicated in the development of

associated symptoms, such as hypersensitivity to visual (Noseda et al. 2010) and auditory stimuli (Filippov et al. 2008).

The major neurotransmitter in the trigeminal ganglion neurons, TCC neurons and third order neurons in the VPM is glutamate (Andreou and Goadsby 2009a). VGLUT1 and VGLUT2 positive neurons in the TCC provide collateral projections to the thalamus (Zhang et al. 2018). In vivo studies using microdialysis and blood flow measurements demonstrated increased levels of glutamate in the TCC during and post stimulation of trigeminal fibres in dural structures (Bereiter and Benetti 1996; Goadsby and Classey 2000). Glutamate plays a crucial role in the transmission of nociceptive information in the VPM. It is involved in signalling from spinothalamic tract and lemniscal pathways and from cortico-thalamic afferents (Broman and Ottersen 1992). Extracellular levels are increased following experimentally produced pain (Silva et al. 2001).

Glutamate triggers post-synaptic excitatory action potentials both in second TCC and third order VPM neurons, by activating multiple glutamate receptors (Andreou et al. 2015; Dougherty et al. 1996; Li et al. 1996; McCormick and von Krosigk 1992; Salt et al. 1999a, b; Salt and Eaton 1995; Salt and Turner 1998). Subunits of all three ionotropic glutamate receptors, namely NMDA, AMPA and kainate receptors, which are involved in fast synaptic signalling, have been found in trigeminal ganglia neurons or on their primary axons on the dura matter (Andreou et al. 2009, 2015; O'Brien and Cairns 2016; Quartu et al. 2002; Sahara et al. 1997; Watanabe et al. 1994). A study in rodents showed that peripherally administrated monosodium glutamate lowers the mechanical threshold of activation of trigeminal fibres in the dura matter, an effect blocked by NMDA receptor antagonists (O'Brien and Cairns 2016). In humans, anecdotal reports exist on the role of dietary monosodium glutamate as a migraine trigger, potentially acting on peripheral glutamate receptors (Borkum 2016; Jinap and Hajeb 2010). Intramuscular injection of glutamate in the masseter muscle which is also innervated by trigeminal fibres evokes pain, potentially through activation of the NMDA receptor (Cairns et al. 2003; Castrillon et al. 2007). These studies further support a role of peripheral glutamate receptors in trigeminal nociception.

On the other hand, earlier brain imaging studies demonstrated increased blood flow in the region of the dorsal rostral pontine and brainstem in both episodic (Afridi et al. 2005; Weiller et al. 1995) and chronic migraine patients (Matharu et al. 2004). The brainstem is known to project a number of descending modulatory circuits to the spinal cord (Akerman et al. 2011), and potentially a malfunction of this modulatory tone may amplify normal sensory processing along the ascending trigeminothalamic pathway (Andreou and Edvinsson 2019).

Within the TCC, microiontophoresis of selective NMDA, AMPA and kainate agonists was shown to excite second order neurons that respond to trigeminovascular stimulation (Andreou et al. 2006; Storer and Goadsby 1999). On the other hand, selective antagonists of NMDA, AMPA and kainate receptors have been shown to inhibit nociceptive trigeminovascular activation of these neurons (Storer and Goadsby 2009a, b), including magnesium, which can block the NMDA receptor (Furukawa et al. 2005). Likewise, within the VPM, agonists of the

ionotropic glutamate receptors were found to excite third order neurons, and selective antagonists were found to inhibit these neurons and trigeminovascular stimulation (Andreou et al. 2008; Salt 2002; Salt and Eaton 1989). Of interest, topiramate, an anti-convulsant approved for the preventive treatment of migraine has been shown to inhibit third order neurons responding to trigeminovascular stimulation, and to selectively block excitation induced by kainate receptor agonists but not by NMDA or AMPA agonists (Andreou and Goadsby 2011).

Some members of the metabotropic glutamate receptors (mGLuRs), which *act by coupling to G-proteins* and modulate differentially activation of sensory fibres, are also found in the trigeminal ganglion. Activation of group I mGluRs (mGluR1 and mGluR5) can increase neuronal excitation through phospholipase C calcium mobilisation (Abe et al. 1992; Pin et al. 2003). Activation of group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8) mGluRs decreases neuronal excitation by inhibiting adenylyl cyclase (AC) resulting in reduction of intracellular cyclic adenosine monophosphate (cAMP) levels (Boye Larsen et al. 2014; Pin et al. 2003; Tanabe et al. 1993). Studies in rats showed that trigeminal neurons express mGluR1α, mGluR2/3 and mGluR8, while satellite glial cells (SGCs) express mGluR1α and mGluR8 (Boye Larsen et al. 2014). The role of mGluRs has been studied extensively in animal models of somatic pain (Pereira and Goudet 2018), however, very few studies investigated their function in trigeminal nociception and in migraine models. The mouse TCC has been shown to express at least the mGluR1, mGluR5, mGluR3 and mGLuR4. These receptors have been also found in the sensory thalamus and in midbrain and medulla sections involved in descending modulation of pain, notably in the periaqueductal grey (PAG) and rostroventral medulla (RVM) (Pereira and Goudet 2018).

Within the thalamus, microiontophoretic studies demonstrated that selective mGLuR1 and mGluR5 agonists can excite third order thalamic neurons (Salt et al. 1999b; Salt and Eaton 1995). Acute thalamic nociceptive responses are found to be mediated by a combination of mGlu1, mGlu5 and NMDA receptor activation, and that co-activation of these receptors produced a synergistic excitatory effect (Salt et al. 1999a; Salt and Binns 2000). On the other hand, agonists that are active at Group II and Group III mGluRs were shown to reduce sensory-evoked synaptic inhibition by a pre-synaptic mechanism (Salt and Eaton 1995; Salt and Turner 1998). A small clinical trial on the acute actions on migraine attack of a selective mGluR5 agonist, ADX-10059, was discontinued due to unacceptable side effects, despite some promise on its efficacy versus placebo (Marin and Goadsby 2010).

The glutamate transporters (GLT) have been also found on trigeminal fibres in the periphery and in the dorsal horn of the TCC (Alvarez et al. 2004; Kim et al. 2015, 2018; Li et al. 2003; Persson et al. 2006). High affinity excitatory amino acid transporters (EAATs) are essential to terminate glutamatergic neurotransmission and to prevent excitotoxicity. So far, five structurally distinct transporters have been identified from animal and human tissues: glutamate/aspartate transporter (GLAST; EAAT1 in human), glutamate transporter-1 (GLT-1; EAAT2 in human), excitatory amino acid carrier-1 (EAAC1; EAAT3 in human), excitatory amino acid transporter 4 (EAAT4) and excitatory amino acid transporter 5 (EAAT5). In the

TCC, it was shown that EAAC1 like-immunoreactivity was present in lamina II. GLAST like-immunoreactivity was also present in lamina II, in both astroglia and neurons and around the central canal (lamina X). GLT-1 was highly expressed in astroglial cells in laminae I-III and the area around the central canal (Tao et al. 2005), and finally, though EAAT4 was initially found to be neuronal in the brain, it has been co-localised with astroglia in the spinal cord (Hu et al. 2003; Rothstein et al. 1994). EAAC1, in addition to its expression in the spinal cord neurons, is detected in dorsal root ganglia (DRG) and distributed predominantly in small DRG neurons (Tao et al. 2005). Some of these EAAC1-positive DRG neurons are positive for CGRP or are labelled by isolectin B4 (Tao et al. 2005), a marker of non-peptidergic neurons. As mentioned earlier, polymorphisms of EAAT2 have been proposed to be involved in the development of chronic migraine (Shin et al. 2011).

Vesicular glutamate transporters (VGLUTs) are considered as the best glutamate markers for staining glutamatergic cells; their presence is a strong indication that glutamate is accumulated in vesicles from which it can be released. Staining studies found these transporters in populations of axons that are known to be glutamatergic and their expression in cultured cells results in glutamate uptake and the subsequent conversion of neurons to a glutamatergic phenotype (Bellocchio et al. 2000; Todd et al. 2003). In the spinal cord both the VGLUT1 and VGLUT2 are expressed, though the spinocervical tract, including the TCC, was found to contain dense labelling for VGLUT2 (Persson et al. 2006). This suggests that VGLUT2 is the transporter responsible for the vesicular accumulation of glutamate at the spinocervical tract terminals, and thus most glutamatergic fibre systems in the spinal cord should display high probability of release, because of their use of VGLUT2 as vesicular transporter (Fremeau Jr et al. 2001; Persson et al. 2006). Spinal, and subsequently TCC, glutamate transporters might play an important role in normal sensory transmission. Intrathecal application of the selective glutamate transporter blocker DL-threo-β-Benzyloxyaspartic acid (TBOA) resulted in significant and dose-dependent spontaneous nociceptive behaviours, and in remarkable hypersensitivity in response to thermal and mechanical stimuli (Liaw et al. 2005). TBOA on the dorsal surface of the spinal cord also resulted in a significant elevation of extracellular glutamate concentrations (Liaw et al. 2005). These findings indicate that a decrease of spinal glutamate uptake can lead to excessive glutamate accumulation in the spinal cord, which might, in turn, result in over-activation of glutamate receptors, and production of spontaneous nociceptive behaviours and sensory hypersensitivity (Tao et al. 2005). However, the glutamate transporters seem to also have opposing actions in pathological pain in animal models. Inhibition or transient knockdown of spinal GLT-1 led to a significant reduction of nociceptive behaviour in the formalin model, whereas different glutamate transporter inhibitors (TBOA, dihydrokainate, threo-3-hydroxyaspartate) reduced formalin-induced nociceptive responses and complete Freund's adjuvant-evoked thermal hyperalgesia (Tao et al. 2005). Different potential mechanisms by which glutamate transporters are involved in pathological pain have been suggested; however, their exact function is not completely understood.

6.4.4 *The Postdrome Phase*

The postdrome phase is the last phase of a migraine attack, which is recognised by at least 80% of patients (Giffin et al. 2016). The postdrome phase occurs after the end of the headache phase and its duration may be between few hours to days. It is mainly characterised by symptoms of fatigue, difficulties in concentration and comprehension, neck stiffness and high disability scores (Giffin et al. 2016). The migraine postdrome is the least studied and least understood phase of migraine. A couple of functional imaging studies showed widespread reduction in brain-blood flow during this phase, but persistent blood flow increase in the occipital cortex (Bose and Goadsby 2016; Schulte and May 2016).

6.5 Pathophysiology of Chronic Migraine and Glutamate Involvement

Chronic migraine is defined by the International Classification of Headache Disorders (ICHD3) of the International Headache Society, as a disorder with headache occurring on at least 15 days per month, which on at least 8 days have the features of a migraine headache (IHS 2018). In chronic migraine it is often impossible to distinguish the individual episodes of headache attacks and the headache appears as a continuous state. About 2.5% of episodic migraine patients progress into chronic migraine (Manack et al. 2011). Chronic migraine is disabling, underdiagnosed and undertreated, affecting about 1–2% of the general population (Buse et al. 2012; Natoli et al. 2010). Factors identified to increase the risk for migraine chronification include de novo increased migraine attack frequency, overuse of acute migraine medication, ineffective acute treatment that could lead to medication overuse, depression and lifestyle factors such as stress, high caffeine intake and obesity (Ashina et al. 2012; Bigal and Lipton 2006; Katsarava et al. 2004; Lipton et al. 2015; Mathew et al. 1990; May and Schulte 2016; Scher et al. 2003).

Chronic migraine appears to induce neuroplastic changes in patients' brain. A number of brain imaging studies showed changes in grey matter volume, as well as in white matter hyperintensities in CM patients, compared to episodic migraine patients (Aradi et al. 2013; Chiapparini et al. 2010; Rocca et al. 2006; Valfre et al. 2008; Zheng et al. 2014), as well as large-scale reorganisation of functional cortical networks and interactive neuronal networks (Coppola et al. 2019). Similar to episodic migraine, cortical excitability appears to be abnormal in chronic migraine patients, but whether this contributes to migraine chronification remains uncertain (Coppola and Schoenen 2012; Cosentino et al. 2014).

The physiological mechanisms that underlie the development of chronic migraine from its episodic form are not understood (Andreou and Edvinsson 2019). However, central sensitization, occurring from peripheral sensitization, has been proposed to play a key role in the development of chronic migraine, similar to other chronic pain

conditions. Central sensitization refers to increased excitability of second order neurons and could even include sensitization of third order thalamic neurons, characterised by increased synaptic strength and enlargement of receptive fields (McMahon et al. 1993; Woolf and Doubell 1994; Woolf and Salter 2000). Central sensitization occurs following repeated activation of peripheral fibres that are at a state of peripheral sensitization leading to the establishment of hyperexcitability in second order neurons in the TCC. Multiple studies in different animal models of pain showed that activity-dependent central sensitization is induced by intense, repeated, or sustained nociceptor inputs. Central sensitization can then persist in the absence of further nociceptor input. Clinically, central sensitization is manifested as a state of either hyperalgesia—an exaggerated pain in response to a stimulus that normally causes mild pain, or of allodynia—a pain response to a normally nonpainful stimulus, and exaggerated pain response referred outside the original pain site (Dodick and Silberstein 2006). Indeed, during a migraine headache about 80% of migraine patients develop cutaneous allodynia, characterised by increased skin sensitivity, mostly within the referred area of pain of the ipsilateral head, but other parts of the body may be also affected, especially if the attack remains untreated (Burstein et al. 2000; Selby and Lance 1960; Su and Yu 2018). Allodynia in non-cephalic areas has been proposed to include sensitization of both second order neurons in the TCC and of third order neurons in the thalamus (Burstein et al. 2000; Dodick and Silberstein 2006). Hence, repeated episodes of peripheral and central sensitization could lead to the development of chronic migraine.

Central sensitization is a glutamate-dependent process and at least, NMDA receptor activation seems to be pivotal for the induction and maintenance of central sensitization in neuronal fibres innervating the dura matter (Woolf and Thompson 1991). Hence, treatment of chronic migraine could target glutamatergic transmission in brain pathways involved in central sensitization, or the peripheral cause in the trigeminal system that induced glutamatergic-driven peripheral sensitization.

Central sensitization requires activation of NMDA receptors for its induction, which leads to elevation in intracellular calcium, activating multiple calcium-dependent kinases that act on receptors and ion channels to further increase synaptic efficacy (Latremoliere and Woolf 2009). AMPA receptors may also participate in the elevation of calcium in the synapse. Studies in multiple pain models suggest that central sensitization includes multiple mechanisms of synaptic plasticity caused by changes in the density, nature and properties of ionotropic and metabotropic glutamate receptors (Latremoliere and Woolf 2009). Ionotropic glutamate receptors can be phosphorylated by intracellular kinases, inducing changes in their activity and trafficking to the membrane, which manifest central sensitization by boosting synaptic efficacy (Carvalho et al. 2000; Lau and Zukin 2007). Stimulation of group I mGluRs also participate, along with NMDA and AMPA receptors, in the activation of the intracellular pathways that sustain central sensitization (Ferguson et al. 2008; Guo et al. 2004; Hu et al. 2007).

In animal models of migraine, inflammatory agents on the dura matter induced long-lasting activation of the trigeminovascular pathway (Burstein et al. 1998; Ebersberger et al. 1997; Schepelmann et al. 1999), which provoked long-lasting

sensitization in trigeminocervical neurons manifested as increased responsiveness and expansion of dural and cutaneous receptive fields (Burstein et al. 1998). These changes were recorded in parallel to an increase of the extracellular glutamate concentration of second order neurons in the TCC (Oshinsky and Luo 2006), indicating an important contribution of glutamate and its receptors in trigeminal allodynia (Oshinsky and Luo 2006). The increased glutamate concentrations in the CSF of chronic migraine patients (Gallai et al. 2003; Peres et al. 2004; van Dongen et al. 2017) indeed support the presence of central sensitization (Burstein et al. 2000).

6.6 CGRP in Migraine and its Modulation of Glutamatergic Transmission

What may initiate peripheral sensitization of the trigeminal nerve that could then lead to the development of central sensitization in chronic migraine remains uncertain, however a role for peripheral inflammation seems plausible (Andreou and Edvinsson 2019; Edvinsson et al. 2019). Calcitonin gene-related peptide (CGRP), of trigeminal origin, is a neuropeptide shown to be increased in the circulation of patients during migraine attacks (Goadsby et al. 1988, 1990) and in between attacks (Ashina et al. 2000). Its levels were shown to be normalised following treatment with sumatriptan (Goadsby and Edvinsson 1993), a $5HT1_{B/D}$ agonist designed as a migraine-specific acute treatment. Intravenous infusion of CGRP has been shown to induce migraine attacks without aura in migraine patients (Hansen et al. 2010). Importantly, CGRP has emerged as a therapeutic target in migraine, since CGRP receptor antagonists and mAbs against CGRP itself or against its receptor are effective preventive treatments for episodic and chronic migraine patients (Andreou et al. 2020; Lambru et al. 2018).

In the peripheral neural tissue, CGRP is found in the trigeminal, dorsal root and vagal ganglia, and their nerve endings, including peri-vasculature nerve terminals in the dura matter (Edvinsson et al. 2020). Centrally, CGRP is found mainly in nerve fibres in the dorsal horn laminae I/IIo of the spinal cord and the TCC, and in some acetylcholine neurons of the ventral horn (Piehl et al. 1991). Small populations of neurons expressing CGRP are also found in the brain (Hokfelt et al. 1992). In migraine animal models, stimulation of trigeminal fibres innervating the superior sagittal sinus increases CGRP circulating levels (Goadsby et al. 1988; Zagami et al. 1990). In humans CGRP levels are increased during stimulation of the trigeminal ganglion, further supporting a trigeminal origin of CGRP in migraine patients (Goadsby et al. 1988).

CGRP is expressed in many human VGLUT1 and VGLUT2 positive trigeminal axons, as well as in rat glutaminase positive neurons (Miller et al. 1993) but not in VGLUT1 positive trigeminal neurons (Cho et al. 2021). However, in immunohistochemistry studies using an anti-glutamate glutaraldehyde antibody in rat and rhesus

monkey trigeminal ganglia found only few neurons co-expressing CGRP and glutamate (Eftekhari et al. 2015). CGRP has been found however to co-release with glutamate, and its release is regulated by voltage-dependent calcium channels (Xiao et al. 2008). Upon its release, CGRP acts on the CGRP receptor which consists of heterodimers of CLR/RAMP1 subunits. Functional CLR/RAMP1 receptors require intracellular interactions with receptor component protein (RCP) and its activation induces stimulation of adenylyl cyclase (AC) and production of *cyclic* adenosine monophosphate (cAMP) (Russell et al. 2014). Recently, the calcitonin receptor CTR/RAMP1 heterodimer (AMY1 receptor) is also believed to be a functional CGRP receptor (Hay et al. 2008; Walker et al. 2015). Functional CGRP receptor(s) have similar distribution patterns as with CGRP neurons and fibres (Russell et al. 2014).

While peripherally, the vascular actions of CGRP as the most potent vasodilator are well characterised (Brain et al. 1985), its modulatory function in somatosensory neurons received considerable attention only recently. Growing evidence indicates that CGRP plays a key role in the development of peripheral sensitization and in the development of neurogenic inflammation. In animals, sustained CGRP release may induce peripheral sensitization of the trigeminal system (Nakamura-Craig and Gill 1991), likely due to the release of pre-synaptic inflammatory mediators, such as bradykinin or prostaglandins from nerve endings and potentiation of post-synaptic glutamate responses (Birrell et al. 1991; Schaible and Schmidt 1988; Wang et al. 2006). CGRP induces release of pro-inflammatory mediators from inflammatory cells (Walsh et al. 2015). Direct application of CGRP on trigeminal fibres on the dura matter does not sensitize second order neurons (Levy 2012; Levy et al. 2005). When CGRP is applied microiontophoretically onto second order neurons in the TCC, in the absence of any other stimulus, it also has little effect on spontaneous neuronal firing (Leem et al. 2001; Miletic and Tan 1988). However, in the presence of glutamate, CGRP can facilitate, inhibit or have no effect on glutamate-evoked firing in second order neurons (Leem et al. 2001; Yu et al. 2002). CGRP was shown to potentiate mainly NMDA, but also AMPA-evoked firing, while in some neurons CGRP showed reciprocal changes, inducing potentiation of NMDA-evoked firing and suppression of AMPA-evoked firing (Leem et al. 2001). Given that CGRP is co-released with glutamate, its role as a glutamatergic modulator is thus of significant importance. Importantly, CGRP can facilitate nociceptive activation of second order neurons and contributes to the development and maintenance of central sensitization (Biella et al. 1991). In animal models of migraine, CGRP antagonists have been shown to inhibit trigeminovascular nociceptive information in parallel to reducing glutamate-evoked activation of second order neurons in the TCC (Storer et al. 2004).

6.7 Current Migraine Treatments Acting as Modulators of Glutamatergic Signalling

Migraine treatment involves acute (abortive) and preventive therapies. Acute treatments are used for relieving migraine headache upon occurrence of a migraine attack. Preventive treatments on the other hand aim to reduce frequency and severity of migraine attacks. A wide range of medications have been used for the preventive treatment of migraine, including beta-adrenoceptor blocking drugs, antidepressants, calcium channel blockers, antiepileptics, botulinum toxin A and the newly developed anti-CGRP mAbs and CGRP antagonists. Acute treatments include triptans, non-steroidal anti-inflammatory drugs, acetaminophen and other over-the-counter pain killers. These treatments exhibit different mechanisms of actions and their efficacy in migraine is variable.

6.7.1 Triptans and Glutamatergic Modulation

Triptans are $5HT1_{B/D}$ agonists specifically developed for the acute treatment of migraine. Part of their anti-nociceptive mechanism of action is believed to be due to the modulation of glutamate release from primary afferents, similar to endogenous serotonin's actions (Travagli and Williams 1996). Triptans have been shown to modulate the release of glutamate from primary afferents in the TCC, by decreasing the amplitude of glutamatergic excitatory post-synaptic currents and reduce the frequency of spontaneous miniature excitatory post-synaptic currents. These actions are potentially mediated by the presence of $5\text{-}HT1_D$ and/or $5\text{-}HT1_B$ receptors on the pre-synaptic terminal, activation of which affects pre-synaptic Ca^{2+} influx (Choi et al. 2012; Hwang and Dun 1999; Jennings et al. 2004). Similar actions of triptans on glutamatergic transmission have been shown in brain neurons (Maura and Raiteri 1996; Stepien et al. 1999), however triptans are unlikely to cross the blood brain barrier (Kaube et al. 1993; Liktor-Busa et al. 2020). Regardless, CSF levels of glutamate in chronic migraine patients who overuse triptans are lower than in CM who overuse other abortive treatments suggesting that triptans mechanism of action may include in part reduction of extracellular glutamate levels in the brain (Vieira et al. 2007).

6.7.2 Preventive Migraine Treatments Acting as Modulators of Glutamatergic Signalling

Topiramate is an anti-epileptic drug used in the preventive treatment of migraine. Its mechanism of action is complicated and rather unclear. Several targets have been proposed to be relevant to the therapeutic activity of topiramate (Aboul-Enein et al.

2012) including voltage-gated sodium channels, high-voltage-activated calcium channels, $GABA_A$ receptors and AMPA/kainate receptors. Although all the above-mentioned mechanisms of action may be relevant to its therapeutic efficacy in migraine, preclinical studies in animal models of migraine showed that topiramate can block nociceptive-evoked activation of second and third order neurons in the TCC and thalamus, respectively (Andreou and Goadsby 2010, 2011; Storer and Goadsby 2004), by selectively blocking kainate agonists-evoked currents (Andreou and Goadsby 2010, 2011). Topiramate's modulatory action on the glutamatergic system may be more complicated as in healthy subjects it was found to increase the cortical levels of glutamine, possibly by acting on the metabolic pathway of glutamate and GABA (Moore et al. 2006). Although topiramate was shown to inhibit CSD in animal models, it was not found effective in preventing migraine aura in patients (Lampl et al. 2004).

Lamotrigine is an anti-convulsant which reduces glutamate release possibly through modulation of voltage-sensitive sodium channels (Lee et al. 2008; Wang et al. 2001). A role for lamotrigine in the prophylactic treatment of migraine has been suggested by small studies, although conflicting outcomes are available (D'Andrea et al. 1999; Gupta et al. 2007; Lampl et al. 1999, 2005; Smeralda et al. 2020; Steiner et al. 1997). Clinically, lamotrigine seems to be an effective treatment option in chronic migraine patients with allodynia, prominent aura and vertigo (Bisdorff 2004; Cologno et al. 2013; D'Andrea et al. 1999).

Ketamine is a medication primarily used for the induction of sedation; however, it can be used for the acute management of pain under controlled conditions (Rocchio and Ward 2021). Ketamine is a non-competitive antagonist at the NMDA receptor which has been used in small studies in migraine patients and found to be effective as an abortive treatment of migraine, especially in patients accessing the emergency department (Bilhimer et al. 2020). It has been also used in FHM migraine with aura patients and found to be effective in reducing the aura symptoms in about 50% of the patients without significant improvement of the migraine headache (Kaube et al. 2000). Ketamine in migraine animal models attenuated neurogenic dural vasodilation (NDV) in rats, demonstrating an additional role for NMDA receptors on the peripheral trigeminovascular system (Chan et al. 2009). Its use has limitations however as its overall efficacy and dosage in relation to the risk of undesirable side effects remain uncertain. Of interest in a small, randomised study, intranasal ketamine was not found to be superior to standard therapy among patients with primary headache syndromes (Benish et al. 2019).

Memantine is another non-competitive NMDA receptor channel blocker which demonstrated significant effects in reducing the headache frequency and the mean disability scores when given as a preventive treatment of refractory migraine (Assarzadegan and Sistanizad 2017; Charles et al. 2007; Krymchantowski and Jevoux 2009; Noruzzadeh et al. 2016; Shanmugam et al. 2019). In a small randomised double-blind placebo-controlled trial memantine was found a tolerable and efficacious preventive treatment in patients with migraine without aura (Noruzzadeh et al. 2016). Its side effects were generally mild (Bigal et al. 2008b; Noruzzadeh et al. 2016). Memantine was shown to inhibit nociceptive

trigeminovascular transmission in second order neurons in the TCC of animal models of migraine (Hoffmann et al. 2019).

Activation of NMDA requires simultaneous binding of both glutamate and the co-agonist glycine (Johnson and Ascher 1987; Kleckner and Dingledine 1988) in conjunction with the removal of Mg^{2+} blockage in a voltage-dependent manner (Mayer and Westbrook 1987). Oral magnesium is commonly used as a non-prescription preventive therapy in migraine (Orr 2016), although appropriate studies providing strong evidence on its efficacy are lacking (Andreou and Goadsby 2009a). It is worth mentioning that Mg^{2+} efficacy may include other mechanisms of action beyond the NMDA receptor. A role for Mg^{2+} in migraine pathophysiology has been suggested as reduced Mg^{2+} levels have been reported in the serum and CSF during and between attacks (Nischwitz et al. 2008; Ramadan et al. 1989; Sarchielli et al. 1992). An older MR spectroscopy study also showed reduced Mg^{2+} concentration within the brain of migraine patients (Ramadan et al. 1989). In animal models of migraine, Mg^{2+} was shown to inhibit nociceptive trigeminovascular transmission in second order neurons in the TCC of (Hoffmann et al. 2019).

Subcutaneous/intramuscular injections of botulinum toxin A (BoNT/A) in the head, neck and shoulders (PREEMPT protocol) is one of the most effective preventive treatments in migraine (Andreou et al. 2018; Aurora et al. 2014; Dodick et al. 2010). BoNT/A cleaves SNAP-25, preventing the correct assembly of the SNARE complex which leads to potent blockade of neurotransmitter and neuropeptide release. At the neuromuscular junction, BoNT/A-induced cleavage of SNAP-25 inhibits the release of acetylcholine from the nerve endings, resulting in muscle paralysis (Binz et al. 2010). Similarly, by cleaving SNAP-25, BoNT/A can interfere with sensory neuronal secretion by blocking pre-synaptic release of glutamate and neuropeptides (Durham et al. 2004; Gazerani et al. 2010; Meng et al. 2009). In animal models of migraine, BoNT/A was shown to block the release of CGRP and of glutamate from trigeminal ganglion neurons (Durham et al. 2004; Gazerani et al. 2010; Meng et al. 2009). In the trigeminovascular model of migraine, BoNT/A was shown to block mechanical activation and sensitization of nociceptors (Burstein et al. 2014; Gazerani et al. 2010). Interestingly, the SNARE complex is also used for the vesicular transport and exocytosis of NMDA and other glutamate receptors on the neuronal membrane (Woo et al. 2020). BoNT/A has been shown to reduce the expression of these receptors, representing an additional anti-nociceptive mechanism of action (Cheng et al. 2013; Woo et al. 2020).

6.8 Future Developments and Perspectives for Glutamate Modulating Treatments

Glutamate is clearly implicated in migraine pathophysiology. Being the major neurotransmitter that drives activation of the ascending trigeminovascular pathway, ultimately a glutamate blocker will be the “cure” of at least the disabling migraine

headache. However, given the abundance of glutamate and its receptors in the brain and their significant function in excitatory neurotransmission, such an option is unacceptable.

Some attempts have been made in the past to block ionotropic glutamate receptors in clinical trials. In a randomised, double-blind, proof-of-concept study which assessed the efficacy of an AMPA receptor antagonist, BGG492, in the acute treatment of migraine, BGG492 was found superior to placebo, but not superior to sumatriptan. However, adverse effects were reported by 80% of patients on the active arm (Gomez-Mancilla et al. 2014). In a randomised double-blind study, a selective GluK1 kainate antagonist LY466195 (Weiss et al. 2006) was effective in relieving acute migraine (Johnson et al. 2008), however, patients reported significant visual side effects. In preclinical studies using selective ionotropic glutamate receptor agonists, Andreou and colleagues identified in addition to kainate, NMDA antagonist actions of this compound on second order neurons in the TCC (Andreou and Goadsby 2009a, b). A possibility is that the visual disturbances reported by patients were mediated by the NMDA antagonism, while both receptors may have participated in the clinical efficacy of this drug. With that in mind, amongst the ionotropic glutamate receptors, the kainate receptor may represent a potential glutamatergic target for future therapeutics, providing that more selective antagonists will become available for clinical trials.

Kainate receptors are not as abundant as NMDA or AMPA receptors, however they are expressed in key structures of the trigeminal nociceptive pathway, including the trigeminal ganglion, trigeminal fibres innervating the dura matter, pre-synaptically and post-synaptically in the TCC and ventroposteromedial thalamus. Their expression within the trigeminal ganglion has been shown to increase after injection of nitroglycerin (Sankaran et al. 2019), which in humans triggers a migraine attack (Iversen and Olesen 1996). In vitro experiments have demonstrated that kainate receptors function as modulators of synaptic transmission and plasticity by regulating post-synaptic currents and pre-synaptic neurotransmitters' release (Andreou and Goadsby 2009a; Bortolotto et al. 1999; Kerchner et al. 2002). Microiontophoretic ejection of selective GluK1 antagonists in the TCC caused a differential response with both inhibition and facilitation in different subpopulations of neurons, activated in response to dural vessel electrical stimulation, by acting at either post-synaptic or pre-synaptic sites (Andreou et al. 2015). Intravenous administration of an iGluR5 agonist inhibited neurogenic dural vasodilation, whereas an antagonist had no effect (Andreou et al. 2009). Direct ejection of an iGluR5 antagonist in the VPM, using microiontophoretic electrode, attenuated activation of third order neurons in response to dural vessel electrical stimulation (Andreou and Goadsby 2009a).

Migraine treatment with metabotropic glutamate receptors has been also attempted in small clinical trials, based on outcomes from preclinical studies on the efficacy of mGluR antagonists in the reduction of hyperalgesia and allodynia in animal models of chronic pain (Fundytus 2001). A potent, selective, negative allosteric modulator of the mGluR5 receptor, ADX10059, was used in small randomised, placebo-controlled clinical trial for the acute treatment of migraine.

ADX10059 showed a statistically significant higher number of patients pain-free 2 h after dosing compared to placebo, however the reported side effects, including hallucinations and vivid dreaming, were discouraging (Marin and Goadsby 2010). Further to this group I mGluR5 antagonist, group II mGluR antagonists have been also advanced into clinical trials in the past for the treatment of acute migraine, however their outcomes have not been published (Johnson et al. 2008). These concerned an mGluR3 antagonist, and a dual mGluR2/cysteinyl-leukotriene 1 (CysLT1) antagonist which entered a Phase II placebo-controlled proof-of-concept study in patients with migraine. This molecule was found to have low brain penetration and was found effective in a preclinical rodent model of migraine, and well tolerated in rat and dog toxicological studies (Célanire et al. 2012). Moving forward, group II mGluRs may still offer a potential therapeutic opportunity, given their important role in pain modulation (Mazzitelli et al. 2018). mGluR2 and mGLuR3 are couple negatively to adenylyl cyclase through Gi/Go proteins, mainly expressed pre-synaptically, and typically their activation has been shown to inhibit the release of neurotransmitters, including glutamate and GABA. Although more knowledge is needed around their function, pharmacological studies in pain models have shown anti-nociceptive effects of group II mGluR agonists, and not of antagonists (Mazzitelli et al. 2018). The availability of orthosteric and new selective allosteric modulators acting on mGluR2 and mGluR3 may provide valuable tools for investigating the role of these receptors in migraine pathophysiology and their potential as therapeutic targets (Mazzitelli et al. 2018).

Any new pharmacological agents that target the glutamatergic system will also have to possess acceptable safety profiles along with clinical efficacy. This is illustrated by the recent discontinuation of clinical trials involving the mGluR5 receptor modulator ADX10059. Perhaps, targeting the glutamatergic system indirectly is an approach that deserves further investigations. For example, the kynurenic acid, a product of the tryptophan-kynurenic pathway, may present such a possibility. Kynurenic acid has been shown to act as an antagonism of NMDA receptors (Stone and Darlington 2002). Studies in chronic migraine patients found altered serum levels of all kynurenine metabolites (Curto et al. 2015). In animal models pre-treatment with kynurenic acid was shown to prevent the nitroglycerine-induced neuronal activation and sensitization in the TCC (Fejes-Szabo et al. 2014), to supress nociceptive activation of the trigeminal pathway (Csati et al. 2015; Lukacs et al. 2016; Veres et al. 2017), and to reduce the release of glutamate (Lukacs et al. 2016). Additionally, the expanding engineering of recombinant BoNT molecules (Dolly et al. 2011), which have been shown to be effective in animal models of migraine (Andreou et al. 2021), could offer in the future the opportunity to selectively block VGLUT1/2 trigeminal fibres, in order to selectively block glutamatergic transmission along the trigeminal system.

Despite the number of studies on pain pathways involved during the headache phase, the molecular changes that actually trigger a migraine attack in the brain remain unknown. The lack of such knowledge had significantly hampered the design of migraine-specific and effective preventive treatments for a long time. Certainly, designing brain acting glutamate modulators could offer a significant therapeutic

value in migraine patients, however any attempt will have to minimise the occurrence of significant side effects, that limited the advancement of glutamate antagonists in migraine clinic.

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Chapter 7
Astrocyte Glutamate Transporter EAAT2 in Alzheimer Dementia

Markku Kurkinen

Abstract Glutamate is an amino acid and also the major synaptic signaling molecule of neurons, essential in cognition, learning, and memory. Glutamate is neurotoxic. As soon as the glutamate signaling starts it is stopped in one millisecond by astrocytes, which take up and clear glutamate from the synapses, and prevent extended glutamate signaling, which can cause synapse loss and neuron cell death. Astrocytes express EAAT2 (excitatory amino acid transporter-2), the major glutamate transporter and 1% of brain protein. In Alzheimer dementia, brain has less EAAT2. In experimental Alzheimer mouse models, decreasing EAAT2 expression enhances dementia progression, and increasing EAAT2 expression slows dementia progression. These and other data indicate EAAT2 as a novel drug target in the treatment and prevention of Alzheimer dementia. In this chapter, after a brief revisit of Alzheimer research and clinical trials, synaptic glutamate signaling and EAAT2, I argue why EAAT2 drugs make therapeutic sense, and then describe a simple drug screening assay how to find them, by targeting the EAAT2 protein reconstituted in liposomes.

Keywords Dementia · Drug discovery · Glutamate · EAAT2 · Synaptic signaling

7.1 Introduction: Alzheimer Dementia

Aging and old age-associated disabilities, diseases, and other geriatric syndromes come hand-in-hand (Franceschi et al. 2018). Many of us know what they are and how to get used to live with them. Yet, nothing compares to *dementia* (*out of mind* in Latin), an unprecedented, ever growing pandemic, and the most devastating disorder

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_7

of the human mind. Alois Alzheimer called it "eine eigenartige Erkrankung der Hirnrinde" (a peculiar disease of the cortex) (Alzheimer 1907).

Alzheimer dementia is detected first by slowly progressing and irreversible memory and mind problems, followed by remarkable behavioral and personality changes, and, in the end, loss of self. Family history of dementia, advanced or old age, is the only high risk factor for developing Alzheimer. These are the risks we cannot do anything about. Other risks include cardiovascular diseases, diabetes, head trauma, obesity, psychiatric symptoms, and stroke (Lane et al. 2018; Qiu et al. 2009; Whitehouse and George 2008). APOE4 is the only major genetic risk factor of Alzheimer (Roses 1996). Some rare early-onset forms of Alzheimer are caused by inherited dominant mutations in APP, PS1, or PS2 gene (Karch and Goate 2015; Tanzi 2013; Van Cauwenbergh et al. 2016). There is no cure or treatment for Alzheimer. There are no Alzheimer survivors.

In the year 1900, in the USA, there were 10,000 people aged 100 years or more. In 2050, there will be 1,000,000. Alzheimer dementia is diagnosed every 3 s, with the prevalence of 10% of the people at age 65, 20% at 75, and 40% at 85. Today 50 million people worldwide have Alzheimer, in 2050 there will be 150 million. In the USA, $1 billion a day goes to health care of 6.08 million people living with Alzheimer at homes or nursing homes (ADI n.d.; Alzheimer's Association 2020; Brookmeyer et al. 2018). In 2020, the National Institutes of Health (NIH) is supporting Alzheimer research with $2.8 billion (www.nia.nih.gov/sites/default/files/2019-07/FY21-bypass-budget-report-508.pdf).

January 4, 2011, in Washington DC, Mr. President Obama signed NAPA (National Alzheimer's Project Act) into law, to "prevent or effectively treat Alzheimer's disease by 2025" (www.govtrack.us/congress/bills/111/s3036/text). December 11, 2013, in London, UK, the world leaders of G8 countries agreed on their "commitment to identify a cure or a disease-modifying therapy for dementia by 2025" (www.alzheimers.org.uk/site/scripts/documents_info.php?documentID=2363&page-Number=4).

A few excellent reviews have described many of the hurdles on the road from drug discovery to clinical development to therapy (Banik et al. 2015; Cummings et al. 2016; Golde 2016). First, it takes time to find the drugs, then study the drugs in preclinical trials on animals, in clinical trials on humans, have the drugs approved by FDA (the US Food and Drug Administration) for human use, and then finally see if the drugs are right for the people living with Alzheimer. This path from drug discovery to Alzheimer therapy is ten or many more years down the road.

Sooner or later there will be safe and cost-efficient drugs for a long-term use by Alzheimer patients. Whether the health care systems and societies at large are adequately prepared for such a time has been a major concern. For example, in their recent commentary *The Edinburgh Consensus*, Ritchie and colleagues (Ritchie et al. 2017) discuss "the implications of disease-modifying treatments for Alzheimer's disease which seem likely to appear in the next few years" and then conclude "The majority of current services in the UK and elsewhere would not be able to accommodate the specialist investigations required to select patients and prescribe these therapies."

7.2 Amyloid Precursor Protein and the Amyloid Hypothesis

Amyloid precursor protein (APP) is a highly conserved protein in 400 million years of evolution (Moir and Tanzi 2019), an indication of multiple molecular interactions of physiologic importance. APP is so named after its proteolytic metabolism, which generates the Aβ peptides which form amyloid, an insoluble aggregate of β-sheet fibrils. First cloned in 1987 by Müller-Hill and colleagues from a brain cDNA library as a 695-amino acid protein, APP has one transmembrane domain, and "features characteristic of glycosylated cell-surface receptors." (Kang et al. 1987).

More recently, APP was found to be a G protein-coupled receptor (GPCR), which binds via its intracellular C-terminal domain to the heterotrimeric G protein subunit Gsα, which activates adenylate cyclase which activates cAMP regulated protein kinase A (PKA) signaling systems, which in turn regulate genes and proteins essential in learning and memory formation. One of them is CREB (cAMP responsive element binding) protein (Deyts et al. 2019).

APP has several isoforms, 639–771 amino acids in length, which differ in tissue and cell expression levels. APP675 is expressed in brain neurons, and APP750 and APP771 are found in most tissues and cells, including leucocytes, erythrocytes, and platelets (Bush et al. 1990; Järemo et al. 2019; Mönning et al. 1990; Müller et al. 2017). APP750 and APP771 have a 56-amino acid domain (inserted after K289) similar to the Kunitz proteinase inhibitor (KPI) (Xu et al. 2017). The proteinase inhibitor called protease nexin-II (PN2) is identical to APP with the KPI-domain, the major APP isoforms in the brain (Van Nostrand et al. 1989). APP and the Aβ peptides play important roles in cerebral hemostasis, capillary blood flow, thrombotic and fibrinolytic events, and hemorrhagic and ischemic strokes (Korte et al. 2019; Van Nostrand 2016).

APP is involved in cancer progression and metastasis. When tumor cell-surface APP binds to death receptor 6 (DR6) on the blood vessel endothelial cells, it induces endothelial cell death, thereby facilitating tumor cell extravasation and metastasis formation (Strilic et al. 2016). Tumor cell-surface APP, but not the soluble extracellular APP domain released from tumor cells, is required for DR6-mediated endothelial cell death. Tumor cells, in which APP expression was reduced by siRNA-mediated knockdown, almost completely lost their ability to form metastases in iv-injected mice. These data agree well with epidemiological studies indicating higher frequency of metastasis formation with increased cancer cell APP expression (Strilic et al. 2016).

The crystal structure of the DR6 ectodomain bound to the E2 domain of APP reveals they interact only at a small interface of 680 $Å^2$ made of hydrophilic and hydrophobic amino acids. Strikingly, a single APP mutation M335K at the interface, whereby hydrophilic lysine (K) replaces hydrophobic methionine (M), prevents APP binding to DR6 (Xu et al. 2015). Small molecule drugs mimicking this interaction could prevent tumor cell-surface APP from binding to DR6 and provide a novel therapy in the treatment of metastatic cancer progression.

In the embryonic brain development, APP is involved in the elimination of synapses, pruning of axons and dendrites, and neuron cell death (Nikolaev et al. 2009). Here, the model of mechanism of action is that after APP and DR6 come together on the neuron cell-surface membrane, APP (as a dimer) binds and induces dimerization of DR6, which then signals death to synapses, dendrites, axons, and neurons (Nikolaev et al. 2009).

Intriguingly, abundant experimental evidence indicates the Aβ peptides as a potent wide-spectrum antimicrobial peptide (AMP), a part of the innate immune system against infections by bacteria, fungi, and viruses (Gosztyla et al. 2018; Moir et al. 2018). As an example, increased expression of Aβ peptides in transgenic mice and *C. elegans* provides increased resistance to infection from both bacteria and viruses. This antimicrobial activity of Aβ peptides is also thought to explain why increased rates of infection have been observed in many participants of Alzheimer drug trials designed to inhibit the production of Aβ peptides and amyloid formation. In contrast, when bacterial or viral brain infection in humans, and experimental animal models, is often associated with elevated amyloid levels, it has been seen as an evidence for microbes causing dementia (Itzhaki 2019).

Overwhelmingly most research on APP, however, has been concerned with the proteolytic products of APP, the Aβ40 and Aβ42 peptides, dominated by the belief that they are the cause of Alzheimer dementia. Proteinases α-secretase and β-secretase cut APP outside the membrane, and γ-secretase cuts APP in the middle of the transmembrane domain. γ-secretase is a complex of four proteins, PS1 (or PS2) as the diaspartyl-proteinase component. APP proteolysis takes place inside the cell, on endosomal membrane, generating the Aβ40 peptide, or less often, the Aβ42 peptide (Ben Halima et al. 2016; Liu et al. 2019; Schreiner et al. 2015; Xie 2019; Yuan et al. 2017).

According to the amyloid hypothesis, Alzheimer dementia begins in the brain with Aβ peptides accumulation, aggregation, and amyloid formation. Therefore, how to stop or slow Alzheimer dementia progression has looked very simple: stop making Aβ peptides. Formulated in 1991–92, the amyloid hypothesis has played a dominant role in Alzheimer research and clinical trials ever since. The amyloid hypothesis is strongly supported by the molecular genetics of the inherited early-onset forms of Alzheimer, which are caused by dominant mutations in APP, PS1, or PS2 gene (Bertram et al. 2010; Hardy and Higgins 1992; Karran and De Strooper 2016; Rosenberg et al. 2016; Selkoe and Hardy 2016). More than 230 mutations, most of them in PS1, have been identified. Overall, the mutations increase, have no effect, or decrease Aβ peptides production. Further, one third of the PS1 mutants are inactive, so cannot produce any Aβ peptides, yet they cause dominant early-onset Alzheimer (Sun et al. 2017). The amyloid hypothesis has been tested in clinical trials, over and over again, and shown to be wrong. Be it with β- or γ-secretase inhibitors to reduce Aβ peptides production, or with anti-Aβ antibodies to clear amyloid from the brain, the trials have failed to stop or slow cognitive decline and memory loss (Anderson et al. 2017; Drachman 2014; Herrup 2015; Kurkinen 2017; Morris et al. 2018).

In 2014, when Jack de la Torre (2014) was writing in *The New England Journal of Medicine*: "The question logically arises: when is a dead hypothesis really dead?" he was commenting on a piece written by Eric Karran and John Hardy (Antiamyloid therapy for Alzheimer's disease: are we on the right road? N Engl J Med 370, 377–378, 2014). Karran and Hardy were reviewing then the high-profile trial failures of anti-Aβ antibodies bapineuzumab and solanezumab, and had said the trials "have provided valuable information" and that the trials of anti-Aβ antibodies should continue.

A popular argument to "explain" the Alzheimer trial failures has been that the drug treatments of mild to moderate Alzheimer dementia patients were "too little too late" to have any therapeutic effect at the time of intervention, when the dementia had already progressed beyond the point of no return. Be as it may, the argument is weak, after the fact, and the logic falls apart with the failures of recent preventive trials. Trials with β-secretase inhibitors of cognitively normal people of age 65–85, at high risk of developing dementia due to APOE4 or elevated brain amyloid PET scan, were all stopped early after 12 months because of serious adverse events and health problems, such as falls and injuries, suicidal ideation, weight loss, sleep disturbance, rash, and hair color change. While the β-secretase inhibitors did reduce Aβ peptides in blood and "dimmed" brain amyloid PET scan, they did not prevent or slow cognitive decline. On the contrary, many study participants showed impaired cognition compared to placebo treated control participants (Egan et al. 2019a, b; Henley et al. 2019; Mullard 2018).

Dominantly Inherited Alzheimer Network (DIAN) is an international registry of families with mutations in APP, PS1, or PS2 gene, the genes that cause 1% of Alzheimer at the early age of 22–55, at about the same age as their mother or father, and their mother or father developed dementia, the exact timing of onset being dictated by the gene and the particular mutation. DIAN people provide an unprecedented, unique opportunity to uncover the cellular mechanisms and molecular details at work many decades before Alzheimer begins (Bateman et al. 2012, 2017; Lopez Lopez et al. 2019; Morris et al. 2012; Ryman et al. 2014). As shown by initial results, the preventive trial by DIAN-TU, explicitly designed not to be "too little too late" (NCT01760005), failed to stop or slow cognitive decline. This was a 5-year trial with anti-Aβ antibodies solanezumab or gantenerumab on presymptomatic people destined to develop dementia due to inherited dominant mutations in APP, PS1, or PS2 gene (Fuller et al. 2019; The New York Times 2020; WUSTL 2020).

Strikingly, a recent case report (Arboleda-Velasquez et al. 2019) describes an individual in Christchurch, New Zealand, with the PS1 mutation E280A, who did not experience mild cognitive impairment (MCI) until her seventies, which is three decades beyond the expected age of 44 of dementia onset with the E280A mutation. She also had two copies of the APOE3 mutation R136S, and an unusually high amount of brain amyloid. Arguably, this case report is a major discovery in dementia research, and as the authors put it "Our findings have implications for the role of APOE in the pathogenesis, treatment and prevention of Alzheimer's disease." (Arboleda-Velasquez et al. 2019).

7.3 Glutamate and Synaptic Glutamate Receptors

Glutamate is an amino acid and also the major synaptic signaling molecule of neurons, essential in cognition, learning, and memory (Danbolt 2001; McEntee and Crook 1993; Watkins and Jane 2006). All cells, except neurons, can synthesize glutamate de novo, for example, from alanine or aspartate, and α-ketoglutarate, a metabolite in the mitochondrial tricarboxylic acid cycle. Neurons make glutamate by converting glutamine to glutamate and ammonia, a hydrolytic reaction catalyzed by glutaminase in mitochondria. Neurons derive glutamine from astrocytes, and the level of glutamate in neurons is adjusted by the glutamine-glutamate cycle between astrocytes and neurons, which maintains the supply for demand in synaptic glutamate signaling.

Brain has the most glutamate in the body, at 5–15 mM, depending on the area, most of it stored in synaptic vesicles at nerve terminals. Other tissues, including blood and cerebrospinal fluid (CSF), have 1000-fold less glutamate. Upon nerve stimulation, glutamate can be released from the nerve terminal vesicles into the synapse (also called the synaptic cleft), where it can bind and activate postsynaptic glutamate receptors, and initiate *neurotransmission*.

Glutamate receptors are ion channels, membrane proteins with one or more transmembrane domains (Reiner and Levitz 2018). Neurons and astrocytes have three types of ionotropic glutamate receptors called NMDA (n-methyl-d-aspartate), AMPA (α-amino-3-hydroxy-5-ethyl-4-isoxazolepropionic acid), and KA (kainic acid) receptor, and five metabotropic G protein-coupled glutamate receptors called mGluR. Of interest, a major difference between the receptors is their signaling time. Ionotropic receptor signaling happens in one millisecond, whereas mGluR signaling can last from seconds to minutes (Reiner and Levitz 2018).

NMDA receptor is the channel for Ca2+ and Na+ inflow and K+ outflow of the cell. NMDA receptor is a tetramer of two GluN1 subunits, and two GluN2 subunits (or occasionally, one or two GluN3 subunits). There are eight GluN1, four GluN2, and two GluN3 subunit variants, which can give rise to many types of NMDA receptors, different in location and regulation of signaling (Paoletti et al. 2013). NMDA receptor is part of a multi-protein complex which includes more than 70 intracellular proteins, such as Neto1, important in the trafficking, stability, composition and function of the NMDA receptor subunits (Ng et al. 2009). Interestingly, GluN3A and GluN3B inhibit NMDA receptor signaling, and GluN2B is necessary for cognition, learning, and memory (Jacobs et al. 2014).

AMPA receptor is a tetramer of two GluA2 subunits, and one or two GluA1, A3, or A4 subunits, each of which binds glutamate. AMPA receptors are the most abundant glutamate receptors, they open and close in one millisecond, provide fast glutamate signaling and mediate most of the synaptic transmission. AMPA receptors with GluA2 prevent Ca2+ inflow, which is proposed to guard against glutamate *excitotoxicity* (Diering and Huganir 2018).

There are five KA receptor subunits, GluK1–5, which form tetramers and channels for Na + and K+ ions. KA receptors play only a minor role in synaptic

transmission. Presynaptic KA receptors regulate glutamate and GABA release, and thus, participate in excitatory and inhibitory synaptic signaling, while activation of postsynaptic KA receptors contributes to synaptic integration. Brain distribution and expression of KA receptors has been linked to schizophrenia, depression, autism, Huntington's, bipolar disorder, and epilepsy, often associated with mutations in GluK subunits (Matute 2011).

7.4 Synaptic Glutamate Signaling

Human brain has 86 billion neurons (19 billion of them in the cerebral cortex), 85 billion glia cells (astrocytes, oligodendrocytes, and microglia), and 1000 trillion synapses (Alonso-Nanclares et al. 2008; Azevedo et al. 2009). As seen in electron microscope, synapses are distinct structures of 100 nm wide, which connect neurons 20–40 nm apart. One neuron can synapse to one or a few other neurons, which in turn can be synapsed by 40,000–140,000 other neurons (Bosch et al. 2015). Astrocytes play an essential role in neural circuit development and synaptic function (Allen and Eroglu 2017; Clarke and Barres 2013; Papouin et al. 2017; Souza et al. 2019; Südhof 2018; Verkhratsky and Nedergaard 2018; Xu and Südhof 2013). Synapses are maintained by cell adhesion molecules, extracellular matrix proteins and proteoglycans, integrins, and remodeled by matrix metalloproteinases (Beroun et al. 2019; Condomitti and de Wit 2018; Park and Goda 2016; Rivera et al. 2019; Smith et al. 2016). Synapses are covered by astrocytes. One astrocyte domain (territory) can cover 270,000to two million synapses (Heller and Rusakov 2015; Oberheim et al. 2009). Synapses are the sites of neurotransmission (Fig. 7.1).

85% of neurotransmission is excitatory, that is, synaptic signaling activates target neurons. Glutamate mediates 95% of excitatory signaling, the remaining 5% is by dopamine, glycine, histamine, and serotonin signaling. Fifteen percent of neurotransmission is inhibitory, synaptic signaling inhibits target neurons. GABA, γ-amino butyric acid (derived from glutamate) is the major inhibitory neurotransmitter. In contrast to excitatory synapses, which target dendrite spines, inhibitory synapses target the neural cell body. In the neural circuit, individual neurons are subject to both excitatory and inhibitory input, synchronized and connected in their activity (Froemke 2015; Sohal and Rubenstein 2019). Brain activity, as studied by EEG or MEG, appears differently synchronized and connected in Alzheimer brains compared to cognitively normal brains, and studies have shown hippocampal hyperactivation in presymptomatic early-onset Alzheimer, as well as in mild cognitive impairment compared to normal aging and Alzheimer dementia (Babiloni et al. 2020; Busche and Konnerth 2015; Dickerson et al. 2005; Quiroz et al. 2010; Wisch et al. 2020; Zott et al. 2018).

Intracellular calcium concentration is 10–20 nM and the extracellular 1–2 mM (Bronner 2003). This 100,000-fold difference generates Ca[2+] electrochemical membrane potential, which powers neurons in synaptic signaling. When an action potential (AP) arrives at the nerve terminal, it can open voltage-gated calcium

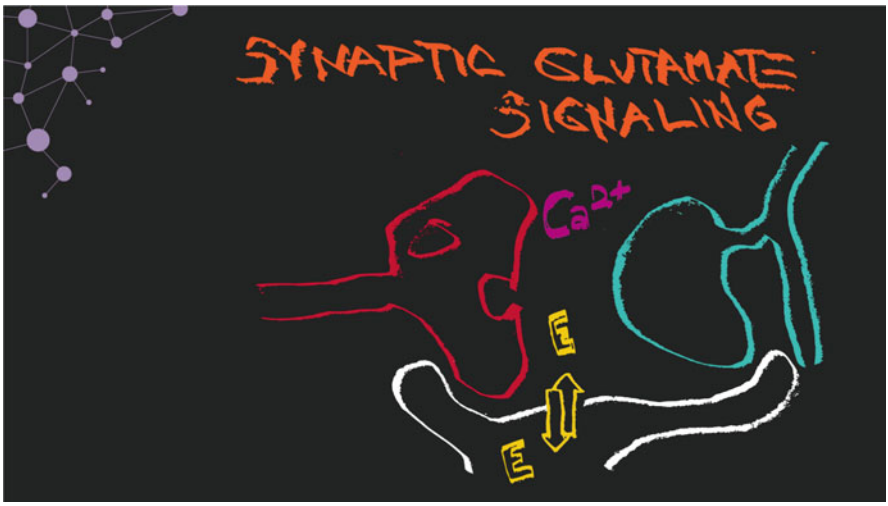

Fig. 7.1 Synaptic glutamate signaling. At the nerve terminal (red), action potential opens membrane channels (not shown) for calcium (Ca2+) inflow, which initiates synaptic vesicle membrane fusion and glutamate (E) release into synaptic cleft. Glutamate binding to synaptic glutamate receptors (not shown) on dendrite spine (green) initiates short-term and long-term calcium-dependent signaling events in the postsynaptic neuron. Glutamate transporter EAAT2 (yellow double arrow) on perisynaptic astrocyte membrane process (white) takes up glutamate from the synapse, and prevents extended glutamate signaling, which causes neurodegeneration. See text for details

channels on the presynaptic membrane for Ca2+ inflow down the concentration gradient, which triggers a membrane fusion reaction with synaptic vesicles, and release of glutamate molecules, 2000/vesicle, into the synapse (Südhof 2013).

In the synaptic cleft of 1 fl (1 um^3), with diffusivity of 0.46 μm^2/ms it takes one microsecond for glutamate to reach the postsynaptic receptors 20–40 nm away (Zheng et al. 2017), Ambient (non-signaling) synaptic glutamate concentration is maintained at 10–20 nM, which goes up to 1–5 mM at the time of glutamate signaling, a 100,000-fold increase. Yet, as soon as the glutamate signaling starts it is stopped in 0.1–2 ms by astrocytes, which take up and clear glutamate from the synapses (Clements et al. 1992; Herman and Jahr 2007; Scimemi and Beato 2009). This fast clearance makes the glutamate signaling essentially an on-or-off event, with a high signal-to-noise ratio, necessary for high-speed neurotransmission with precision. Fast glutamate clearance also prevents extended glutamate receptor signaling, excessive calcium inflow and calcium signaling, which in turn can impair synaptic structure and function, cause synapse loss, and in the end, neuron cell death.

Synaptic NMDA receptor activation begins by D-Serine (secreted by astrocytes and neurons) binding to GluN1, and glutamate binding to GluN2. In addition, it requires simultaneous glutamate binding to AMPA receptor, and inflow of Na + ions, which *depolarize* the postsynaptic membrane potential, make it more positive-inside, which repels Mg2+ ion (the natural NMDA receptor inhibitor) from the GluN2 subunit, thereby opening the channel for Na+ and Ca2+ inflow, and K+

outflow, down their membrane gradients (Vyklicky et al. 2014). The outcome of all of this is a more depolarized postsynaptic neuron able to generate an EPSP (excitatory postsynaptic potential). EPSP is not an "all-or-nothing" type of action potential, but fades away as it travels to the neural cell body, the soma. However, when many of these excitatory potentials, produced locally at a high rate or globally at many different synapses, spines and dendrites, reach the soma at about the same time, together they can generate a true action potential at the "axon-hillock" (Leterrier 2018).

Long-lasting effects of calcium entry begin with Ca2+ binding to calmodulin (CaM), which binds and activates CaMKII (calcium/calmodulin regulated kinase II) and CaN (calcineurin) phosphatase, which in turn regulate many signaling systems in the postsynaptic neuron (Clapham 2007; Rusnak and Mertz 2000; Yamaguchi 2005). For example, regulation of the number and type of glutamate receptors determines synaptic "strength" measured by LTP (long-term potentiation) and LTD (long-term depression), which are generally thought to be the electrophysiologic basis of learning and memory (Baltaci et al. 2019; Diering and Huganir 2018; Lynch 2004; Malenka and Bear 2004). In LTP, more AMPA receptors make synapses stronger, facilitate glutamate signaling, and enhance neurotransmission. In LTD, with less AMPA receptors, the opposite is true.

The loss of synapses in hippocampus and neocortex is an early event in the development of Alzheimer dementia, and the best correlate of cognitive impairment (Blennow et al. 1996; Colom-Cadena et al. 2020; Terry et al. 1991). Scheff and colleagues have estimated the number of synapses, by electron microscope, in the outer molecular layer of the dentate gyrus in postmortem autopsy tissue of individuals with early Alzheimer dementia (eAD), mild cognitive impairment (MCI), or no cognitive impairment (NCI). Individuals in the eAD group had significantly fewer synapses than individuals in the MCI and NCI groups. Seventy five percent in the MCI group had fewer synapses compared to the NCI group. Synaptic numbers correlated with the individual's Mini-Mental State Examination (MMSE) score, but showed no correlation with APOE genotype or Braak stages of Alzheimer. Indeed, as the authors put it "This study supports the concept that synapse loss is an early event in the disease process and suggests that MCI may be a transition stage between eAD and NCI with synaptic loss [as] a structural correlate involved in cognitive decline." (Scheff et al. 2006).

Török and colleagues (Helassa et al. 2018) have constructed an ultrafast glutamate sensor (iGlu*u*), a genetically engineered green fluorescent protein to image glutamate at individual synapses of choice. In rat hippocampal slice culture stimulated at 100 Hz, iGlu*u* was fast enough to resolve individual glutamate release events, that is, in every 10 ms. This result clearly shows how rapidly glutamate can be cleared from the synapse ex vivo in time.

Transgenic Q175 mouse is a model of Huntington disease. Q175 mice express less EAAT2 around corticostriatal nerve terminals. Synaptic glutamate imaging with iGlu*u* revealed prolonged glutamate clearance in the Q175 mice. Treatment of wild type mice with the EAAT2 inhibitor TFB-TBOA mimicked the prolonged glutamate clearance seen in the Q175 mice. As the authors write "The results provide a positive

answer to the hitherto unresolved question of whether neurodegeneration (e.g., Huntington's disease) associates with a glutamate uptake" and then suggest "... astrocytic Glu transport remains a promising target for therapeutic intervention ..."(Dvorzhak et al. 2019).

7.5 Glutamate Is Neurotoxic

In 1957, Lucas and Newhouse showed that high blood glutamate level caused mice to lose their sight due to retinal cell death (Lucas and Newhouse 1957). Twelve years later, John Olney (Olney 1969) reported that mice treated with monosodium glutamate (MSG) showed neuron cell loss in brain areas not protected by the blood–brain barrier, developed obesity and other disturbances, a phenomenon he called [glutamate] *excitotoxicity*. In 1989, Rosenberg and Aizenman demonstrated "Hundred-fold increase in neuronal vulnerability to glutamate toxicity in astrocyte-poor cultures of rat cerebral cortex" (Rosenberg and Aizenman 1989). In 1992, Rosenberg and colleagues did a simple experiment with an astrocyte-rich neuron cell culture, but without sodium in the culture medium. Astrocyte glutamate uptake was impaired, to the effect, that they were no longer able to protect neurons from dying of glutamate excitotoxicity (Rosenberg et al. 1992).

7.6 Astrocyte Glutamate Transporter EAAT2

Humans have five glutamate transporters, also called excitatory amino acid transporter (EAAT), which differ by tissue and cell distribution, sub-cellular level of expression, and glutamate uptake kinetics (Murphy-Royal et al. 2017; Olivares-Bañuelos et al. 2019; Vandenberg and Ryan 2013). One percent of brain protein is EAAT2, which covers 95% of synaptic glutamate uptake. Astrocytes express most of the EAAT2 protein. By electron microscope studies, 90% of EAAT2 is found on the perisynaptic astrocyte membrane processes around synapses, at the density of 8500/um2, or 25,000 per synapse (Danbolt 2001; Roberts et al. 2014).

EAAT2 of 573 amino acids is encoded by 34 Mb gene with 11 exons on chromosome 11. There are several splice and exon-skipping variants of EAAT2 (Gebhardt et al. 2010; Scott et al. 2011). EAAT2 is 36% identical in amino acid sequence with the aspartate transporter GltPh from *Pyrococcus horikoshii* bacterium, however, most of the amino acids indicated in glutamate binding and transport are conserved. EAAT2 has eight transmembrane (TM) domains, two helical hairpins HP1 and HP2, a trimerization domain and the glutamate transport domain (made of TM7, HP2, and TM8). In the crystal structure, GltPh appears as trimer (Akyuz et al. 2013; Yernool et al. 2004). GltPh provides the best model for how EAAT2 works. As seen in the movies made by Ryan and colleagues (Ruan et al. 2017), using high-speed atomic force microscopy, EAAT2 works like an elevator. When one glutamate

molecule, three Na+ ions and one H+ "proton" bind to each of the three glutamate transport domains, they separately and independently can go down the membrane 15 Å (1.5 nm), one third of the membrane, and rotate 35 degrees against the trimerization domain, which stands still in the membrane. After dropping off glutamate, Na+ and H+ inside the cell, one K+ ion binds to the "empty," glutamate-free, transport domain, which *isomerizes,* goes up the membrane, and resets EAAT2 for another glutamate binding and transport. An EAAT2 mutant E404D, which cannot bind K+ ion, cannot isomerize, be exposed to the outside, and therefore cannot transport glutamate (Kavanaugh et al. 1997).

The rate-limiting step in glutamate uptake is the glutamate-bound EAAT2 moving down the membrane, which takes 50 ms, while EAAT2 without glutamate moving up happens in 0.5 ms (Akyuz et al. 2013). This immediately raises the question: how can astrocytes and EAAT2 handle all that synaptic glutamate signaling, a 1 ms event? It seems that, just by binding glutamate, EAAT2 can easily clear synaptic glutamate, even at the rapid synaptic firing rate of 100 Hz (Helassa et al. 2018). Indeed, as of 1% brain protein, and 25,000 copies on astrocyte membrane processes around synapses, EAAT2 is exactly where it should be,

7.7 Regulation of EAAT2 Expression: Drugs

In 2005, Rothstein and colleagues screened 1040 FDA-approved drugs and nutritionals to look for regulation of EAAT2 expression. Their assay was made of spinal cord slice cultures prepared from 9-day-old rats (to preserve cell–matrix interactions and metabolism ex vivo). One of the drugs they identified was *ceftriaxone* (*CEF*), a potent wide-spectrum β-lactam antibiotic. *CEF* increased EAAT2 expression by gene transcription. In mice, *CEF* increased EAAT2 expression in the brain, suggesting C*EF* entered the brain. *CEF* was neuroprotective in cell culture models of ischemic brain injury and motor neuron cell death (Rothstein et al. 2005). In a mouse model of focal cerebral ischemia, pretreatment with *CEF* reduced infarct volume and improved recovery. These effects of *CEF* were not seen if EAAT2 was inhibited with dihydrokainate (DHK), a specific inhibitor of EAAT2 (Chu et al. 2007).

Amyotrophic lateral sclerosis (ALS) is caused by motor neuron cell death associated with astrocyte impairment, reduced EAAT2 expression and glutamate uptake (Rosenblum and Trotti 2017; Rothstein et al. 1992). As shown by Rothstein and colleagues using the transgenic SOD1(G93A) mice, a model of ALS, *CEF* delayed motor neuron cell death and loss of muscle strength, and increased survival time by 10 days (Rothstein et al. 2005). A human ALS trial with *CEF* (clinicaltrials.gov NCT00349622) had to be stopped early because *CEF* had little or no effect, except causing adverse side effects on the gastrointestinal and liver function. There was no increase in survival time (Cudkowicz et al. 2014).

Rats treated with 6-hydroxydopamine is a model of Parkinson disease. In these animals, EAAT2 expression was decreased in striatum, whereas neural glutamate

transporter EAAC1 expression was increased in the *substantia nigra pars reticulata*. Increased EAAC1 was thought to increase GABA synthesis (from glutamate) and enhance inhibitory synaptic GABA signaling (Chung et al. 2008). In another study with 6-hydroxydopamine-treated rats, *CEF* increased EAAT2 expression and prevented dopamine neurons from dying. In behavioral tests, grip strength was increased and numbers of apomorphine-induced contralateral rotation were reduced (Leung et al. 2012). OXYS rat strain is a natural model of accelerated senescence. In these rats, *CEF* was neuroprotective and improved cognition and behavior (Tikhonova et al. 2017).

Wistar male rats, stereotaxically injected with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) into *substantia nigra pars compacta* (SNc), is another model of Parkinson disease. These rats develop impaired motor function, decreased working memory and object recognition, and in addition, have reduced neurogenesis in SNc and dentate gyrus of the hippocampus. *CEF* prevented all of these behavioral, cognitive, and neuronal defects (Hsieh et al. 2017).

Clearly, these and other studies in cell culture and animal models of a variety of different neurodegenerative diseases indicate *CEF* as a potential drug to stop or slow the disease progression (Yimer et al. 2019). *CEF* increases EAAT2 expression by gene transcription via the nuclear factor-kappa-B (NFκB) signaling pathway. NFκB binds to the EAAT2 promoter at the −272 position (Lee et al. 2008).

LDN/OSU-0212320 (*LO*) is a small molecule translational activator of EAAT2, that is, *LO* increases EAAT2 expression by mRNA translation. It was discovered after studying a library of 140,000 small molecules in a cell-based high-throughput screening (HTS) assay designed to measure EAAT2 mRNA translation (Colton et al. 2010). In astrocyte-neuron cell culture, *LO* protected neurons from glutamate excitotoxicity and death by increasing EAAT2 expression. This effect was not seen if EAAT2 was inhibited with DHK. In the SOD1 (G93A) mice, a model of ALS, *LO* delayed motor function decline and extended lifespan. *LO* also reduced mortality, neuronal cell death, and spontaneous recurrent seizures in a pilocarpine-induced model of temporal lobe epilepsy (Kong et al. 2014).

Parawixin-1 is a neuroprotective compound from *Parawixia bistriata* spider venom. As originally studied by dos Santos, Fontana, and Amara, it increases astrocyte glutamate uptake (Fontana et al. 2003; Fontana et al. 2007). *Parawixin-1* is EAAT2 specific, it increases glutamate uptake 70% by COS-7 cells expressing EAAT2 but not by COS-7 cells expressing EAAT1 or EAAT3. Most important, *parawixin-1* does not increase glutamate uptake by increasing EAAT2 expression, but by increasing EAAT2 activity, as shown by glutamate uptake by EAAT2 reconstituted in liposomes. Recently, Fontana and colleagues have found, after a virtual screening of two million chemical compounds, three drugs that increase glutamate uptake by astrocytes, and EAAT2 liposomes (Kortagere et al. 2017). One of them called *GT949*, added to an astrocyte-neuron cell culture medium, protected neurons from dying of glutamate excitotoxicity (Falcucci et al. 2019).

Rapamycin (sirolimus) is an immunosuppressant drug, most often used in kidney transplants. *Rapamycin* inhibits mTOR (mammalian target of rapamycin), which is a serine/threonine protein kinase that regulates interleukin-2 activation of T

and B cells. In the MPTP-treated rats, a model of Parkinson disease, *rapamycin* increased EAAT2 expression and prevented dopamine neurons from dying (Zhang et al. 2017).

Raloxifene (*Evista*) is a selective estrogen receptor modulator (SERM) used in invasive breast cancer treatment, and in the management of osteoporosis. In breast, *raloxifene* inhibits, and in bone, *raloxifene* activates the estrogen receptor (Clemett and Spencer 2000; Moen and Keating 2008). Interestingly, *raloxifene* improved verbal memory of women aged 70 and over, and reduced dementia risk in women with osteoporosis (Jacobsen et al. 2010; Yaffe et al. 2005). Osteoporosis is a dementia risk (Chang et al. 2014; Zhou et al. 2014). As shown by Karki and colleagues, *raloxifene* increased EAAT2 expression by gene transcription by activating many signaling systems involving EGFR, MAPK, PI3K/Akt, *Src*, CREB, and NFκB (Karki et al. 2014). Clearly, this wide signaling spectrum contraindicates *raloxifene* in general use as a "memory" drug.

For many additional drugs, EAAT2 expression, and relevant diseases, please see the excellent review by Andréia Fontana (Fontana 2018).

7.8 EAAT2 in Alzheimer Dementia

In okadaic acid-induced rat model of Alzheimer dementia, increasing EAAT2 expression with *CEF* reduced short- and long-term memory impairment, as measured by behavioral and passive avoidance tests. *CEF* also significantly reduced attenuation of field excitatory postsynaptic potential (fEPSP) slope and population spike (PS) amplitude indicating its beneficial effects on both short-term and long-term synaptic plasticity (Hamidi et al. 2019).

Transgenic 3xTg mice, a model of Alzheimer, express less EAAT2 in the hippocampus. In these mice, increasing EAAT2 expression with *CEF* significantly restores synaptic proteins, prevents cognitive decline, reduces the age-dependent *tau* accumulation, but has no effect on brain amyloid (Hamidi et al. 2019). The authors also show that Aβ peptides down regulate EAAT2 expression in astrocyte-neuron cell culture. These results suggest that EAAT2 is neuroprotective, and that reduced EAAT2 expression caused by Aβ peptides may serve as one of the "pathological links between Aβ and tau pathology" (Fan et al. 2018).

In transgenic APP/PS1 mice, a model of Alzheimer, *CEF* increased EAAT2 expression in the hippocampus and significantly reduced cognitive deficits, as measured by Morris water maze test. *CEF* also increased the expression of glutamine synthetase (GS) and system N glutamine transporter 1 (SN1) of the glutamate-glutamine cycle. *CEF* had none of these effects if EAAT2 and glutamate uptake was inhibited with dihydrokainate (Zumkehr et al. 2015).

In transgenic APPswe,Ind mice, a model of Alzheimer, increasing EAAT2 expression with *LDN/OSU-0212320* improved "cognitive functions, restored synaptic integrity, and reduced amyloid plaques" (Takahashi et al. 2015a). Even after stopping the drug treatment, the effects were observed for 1 month, which made the

authors write: “EAAT2 is a potential disease modifier with therapeutic potential for AD [Alzheimer dementia].” (Takahashi et al. 2015a).

Riluzole, an FDA-approved drug, has been used in the management of ALS since 1995. *Riluzole* delays the onset of ventilator-dependence (tracheostomy) in some people and may increase survival time by 2–3 months (Fang et al. 2018). *Riluzole* decreases presynaptic glutamate release, increases EAAT2 expression, and improves memory (Pereira et al. 2017; Vallée et al. 2020). A comparison of Alzheimer patients data with the effects of *riluzole* in aged rats revealed that many of the gene expression changes observed in Alzheimer are reversed in *riluzole* treated rats. RNA-sequencing and immunohistochemistry confirmed increased EAAT2 expression in hippocampus, identifying a possible mechanism for the improved memory of *riluzole* treated old rats (Pereira et al. 2017). In transgenic APPswe/PS1dE9 mice, a model of Alzheimer, early life stress (ELS) impairs synaptic plasticity and spatial memory, in close correlation with reduced hippocampal EAAT2 expression. In *riluzole* treated mice, ELS had none of these effects (Lesuis et al. 2019). These and other data suggest a potential role for *riluzole* in the treatment and prevention of Alzheimer dementia (Vallée et al. 2020).

When APPswe/PS1dE9 mice where crossed with transgenic mice with only one copy of the EAAT2 gene, the crossed mice showed increased spatial memory problems at 6 months and behavioral disorders at 9 months. These results suggest that reduced synaptic glutamate uptake (because of reduced EAAT2 expression from only gene) enhances the progression of dementia caused by APP and PS1 mutations (Mookherjee et al. 2011).

When APPswe,Ind mice (which express 40% less EAAT2 in the brain) were crossed with transgenic EAAT2 mice expressing two-fold more EAAT2, EAAT2 expression was normalized in the crossed mice, which also showed improved “cognitive functions, restored synaptic integrity, and reduced amyloid plaques” (Lin et al. 2012). In astrocyte-neuron cell culture, prepared from the transgenic EAAT2 or wild type mice, $A\beta_{25–35}$ oligomers decreased EAAT2 expression 5% in transgenic, and 45% in wild type culture. The EAAT2-specific, dihydrokainate-sensitive, [^{3}H]glutamate uptake decreased 16% in transgenic, and 60% in wild type culture. When neuron cell morphology was visualized using MAP 2 antibody immunostaining, $A\beta_{25–35}$ oligomers caused profound damage and neurite degeneration in wild type cultures, whereas neurons in transgenic EAAT2 cultures were spared. These results suggest that increased EAAT2 expression protects against neuronal damage by $A\beta_{25–35}$ oligomers (Lin et al. 2012).

Conditional deletion of EAAT2 in mice leads to early deficits in short-term and long-term memory, and in spatial reference learning (Sharma et al. 2019). Interestingly, EAAT2 deficiency also results in impaired innate and adaptive immune pathways, which correlate with cognitive decline. Furthermore, in these mice, gene expression changes associated with inflammation and synaptic function appear similar to those observed in the aging human brain and Alzheimer dementia (Sharma et al. 2019).

In a study of midfrontal cortex of normal and Alzheimer brains, EAAT2 expression, measured by [3H]aspartate binding, was compared to synaptophysin levels,

brain spectrin degradation products, and other clinical and neuropathological indicators (Masliah et al. 1996). Compared to control brains, Alzheimer brains had 30% decrease in [3H]aspartate binding, 48% loss of synaptophysin, and increased levels of brain spectrin degradation products. These results suggest that decreased EAAT2 activity in Alzheimer dementia is associated with increased excitotoxicity, synaptic damage, and neurodegeneration (Masliah et al. 1996).

As shown by Jacob and colleagues (Jacob et al. 2007), in a study of EAAT1, EAAT2, and glutamate receptors in Alzheimer brains, EAAT1 and EAAT2 gene and protein expression were reduced already in the early stages of Alzheimer dementia, in hippocampus and gyrus *frontalis medialis*. The loss of EAAT1 and EAAT2 proteins was particularly obvious in the vicinity of amyloid plaques. In later dementia stages, KA (GluK4) receptor was upregulated, and AMPA (GluA4) and NMDA (GluN1A) receptors were downregulated. This study supports the causal role of impaired synaptic glutamate uptake and excessive glutamate signaling in the pathogenesis of Alzheimer dementia (Jacob et al. 2007).

In a study of EAAT2 mRNA splice variants in Alzheimer brains (freshly obtained at autopsy), wild type EAAT2 mRNA showed global reduction, while mRNA splice variant for EAAT2b (563 amino acids), specific to perisynaptic astrocyte processes, showed no significant variation. Remarkably, brain regions vulnerable to neuronal loss demonstrated greater expression of mRNA splice variants for EAAT2 with reduced activity, as shown by an in vitro assay of glutamate uptake. As the authors, Dodd and colleagues, put it "These results have implications for the treatment of AD as modulators of EAAT2 splicing and/or glutamate uptake would augment current therapies aimed at blocking glutamate receptors" (Scott et al. 2011).

In contrast to all these data on EAAT2 in Alzheimer dementia, when Garcia-Esparcia et al. (Garcia-Esparcia et al. 2018) studied EAAT2 mRNA expression, by real time quantitative PCR (RT-qPCR), in postmortem human brain samples of frontal cortex Brodman area BA8 in advanced stages of Alzheimer and terminal stages of dementia with Lewy bodies (DLB), they found no difference between the samples, and no difference from the normal brains.

The best proof of concept for the role of EAAT2 in the development of dementia is provided by HIV, the virus that causes AIDS. HIV also causes HIV-associated neurocognitive disorder (HAND), also called HIV-associated dementia (HAD) (Clifford and Ances 2013; Heaton et al. 2010). In the brain, HIV infects astrocytes and microglia cells, but not neurons. In astrocytes, HIV envelope glycoprotein gp120 inhibits EAAT2 gene transcription and glutamate uptake (Potter et al. 2013; Rumbaugh et al. 2007; Wang et al. 2003). Increasing EAAT2 expression with *CEF* protects against HIV neurotoxicity (Rumbaugh et al. 2007). Of note, as reported by Togas and colleagues in 1994, transgenic mice expressing gp120 in astrocytes develop neurodegeneration (Toggas et al. 1994). Transgenic HIV Vpr mice, a model of HAND, express less EAAT2 in the brain (Power et al. 2012). Further, HIV-infected people being treated with a combination antiretroviral therapy, which includes two HIV proteinase inhibitors, amprenavir (APV) and lopinavir (LPV), often experience cognitive and behavioral problems (Underwood et al. 2015). As reported by Vivithanaporn and colleagues, APV and LPV inhibit EAAT2 expression

and glutamate uptake in astrocytes in cell culture. In addition, APV or LPV treated HIV Vpr mice show impaired learning and memory, associated with less EAAT2 expression in the brain (Vivithanaporn et al. 2016).

7.9 EAAT2 Drugs: The Liposome Glutamate Assay

To discover drugs, chemical compounds and natural products that can activate EAAT2 and increase glutamate uptake, we have designed a simple assay, which targets the EAAT2 protein reconstituted in lipid vesicle (liposome) membrane, and measures glutamate uptake by red light (Fig. 7.2). In brief, *Oxonol VI* is a negatively charged lipid soluble molecule, which can diffuse in and out of liposomes. When *Oxonol VI* binds to liposome membrane lipids, its excitation and emission wavelengths increase 10–15 nm. This "red shift" makes it possible to measure lipid-bound *Oxonol VI* in liposomes (Apell and Bersch 1987; Apell and Damnjanovic 2016). EAAT2 is electrogenic and glutamate transport is powered by a Na[+] membrane gradient (Grewer and Rauen 2005), that is, with every glutamate molecule, three Na[+] ions and one H[+] "proton" are co-transported inside. Drugs that activate EAAT2 and glutamate uptake make the liposomes more positive-inside, to the effect, that more *Oxonol VI* stays inside the liposomes, binds to the membrane lipids, and emits more 660 nm red light (when excited at 580 nm yellow light).

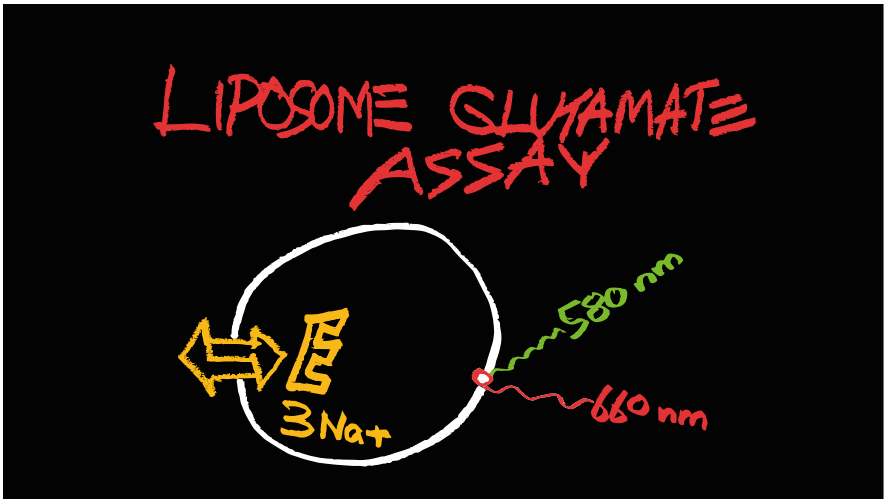

Fig. 7.2 Liposome glutamate assay. EAAT2 (yellow double arrow) reconstituted in liposome membrane (white) transports glutamate (E) and three sodium (Na+) ions, which make the liposome more positive-inside. *Oxonol VI* (red circle) is a negatively charged red fluorescent molecule, which diffuses in and out of liposome. Excited at 580 nm, membrane lipid-bound *Oxonol VI* fluorescences at 660 nm. More positive-inside liposome retains more *Oxonol VI* inside and emits more 660 nm light. In other words, the more glutamate in, the more red light out. See text for details

7.10 Why Target EAAT2 in Liposomes?

The answer is simple: it is the best drug screening assay we can think of because it targets nothing but EAAT2. The assay is easy to do, fast, unbiased, blind, and cost-effective. Formatted in a high-throughput screening (HTS) platform, drugs, chemical compounds and natural products can be screened in a rather short time. Furthermore, consider this:

1. If a drug increases liposome glutamate uptake, then we know the drug must directly activate the EAAT2 protein. In contrast, in a drug screen targeting EAAT2, for example on cells in culture, it would not be possible to target EAAT2 anywhere near with the same precision. In a cell-based assay, a drug could increase glutamate uptake by increasing the amount of EAAT2 on cell-surface membrane, by increasing EAAT2 gene transcription, mRNA translation, protein targeting and trafficking, or by some other unknown mechanism. These mechanisms cannot be EAAT2 specific, indeed, cell signaling systems are never private, but always shared in many molecular details, so such a drug would also act on other targets, and cause side effects and adverse events.
2. An argument could be made for proteins as the best targets in drug discovery, because the 3D structure of proteins has a more "rich landscape" for specific drug interactions, in contrast to the 1D or 2D landscapes displayed by DNA and mRNA.
3. In glutamate uptake, EAAT2 reconstituted in liposomes works the same way as it does in astrocytes in cell culture, as measured by similar Vmax and Km values. This suggests a self-autonomous mechanism of action of EAAT2, with no additional cellular components needed. This is very important because it implies that drugs acting on EAAT2 in liposomes can do the same on EAAT2 in astrocytes.

7.11 In Perspective

As Hans-Christian Danbolt has said "Like glutamate itself, glutamate transporters are somehow involved in almost all aspects of normal and abnormal brain activity" (Danbolt 2001). A number of papers and reviews of literature indicate EAAT2 as a novel target for drug discovery and clinical development in the treatment and prevention of a variety of cognitive, psychiatric, neurodevelopmental, and neurodegenerative disorders, such as ADHD, drug addiction, alcoholism, ALS, Alzheimer dementia, autism, depression, bipolar disorder, epilepsy, glioblastoma, Huntington disease, migraine, chronic pain, Parkinson disease, schizophrenia, ischemic stroke, and essential tremor (Ayers-Ringler et al. 2016; Blacker et al. 2020; Chu et al. 2007; Fontana 2015; Fontana 2018; Gegelashvili and Bjerrum 2019; Ghanizadeh and Berk 2015; Hubbard and Binder 2017; Jia et al. 2017; Kurkinen 2018; Laprairie et al. 2019; Lee et al. 2011; Mookherjee et al. 2011; Naaijen et al. 2017; Nanitsos et al.

2005; O'Donovan Sinead et al. 2017; Pajarillo et al. 2019; Parkin et al. 2018; Petr et al. 2015; Rao et al. 2015; Rojas 2014; Takahashi et al. 2015b; Wang et al. 2016; Wei et al. 2019; Yang et al. 2018; Zhou et al. 2019).

Drug discovery and clinical development is a very expensive experiment that can last 10–15 years, yet fails most of the time (Banik et al. 2015; Batool et al. 2019; Cummings et al. 2016; Gisbert Schneider 2018; Golde 2016). Ninety-nine percent of drugs in the pipeline of drug industry never reach the FDA-approved standard for marketing in human use. A major reason for this is the lack of efficacy, specificity, safety, and adverse side effects of the drugs, or "noise" as it is called in the drug industry parlance.

Alzheimer is diagnosed every 3 s. Today 50 million people live with Alzheimer at homes or long-term care facilities. In the past, Alzheimer drug trials targeting brain amyloid have been unsuccessful, and it should be absolutely clear by now, trials based on the amyloid hypothesis will be unsuccessful.

In this chapter, I have presented EAAT2 as an important novel drug target in the treatment and prevention of Alzheimer dementia, and have described a simple assay using EAAT2 reconstituted in liposomes to find drugs that increase glutamate uptake. The assay is blind, not structure-based, and assumes nothing about the mechanism of action of EAAT2. The drugs could act on EAAT2 outside or inside the membrane, the drugs could act on membrane lipids regulating EAAT2 activity (McIlwain et al. 2015), or the drugs could act by some other unknown mechanisms. In short, we are looking for drugs that make the glutamate-bound EAAT2 go down faster in the membrane, because that is the rate-limiting step in glutamate uptake. Indeed, the first thing to do to regulate a chemical reaction is to find out the rate-limiting step, and then find out ways to regulate that step. Is there a rate-limiting step in the development of Alzheimer dementia?

Why, and how, is EAAT2 expression downregulated in Alzheimer dementia? EAAT2 expression is subject to several signaling systems regulating transcription, transcript splicing, mRNA processing and translation, protein trafficking and targeting, and finally, EAAT2 activity (Abdul et al. 2009; Alam and Datta 2019; Fontana 2015; Ghosh et al. 2016; Huerta et al. 2006; Karki et al. 2014; Laprairie et al. 2019; Lauriat and McInnes 2007; Lutgen et al. 2016; Martinez-Lozada et al. 2016; Underhill et al. 2015; Vallée et al. 2020). For example, EAAT2 gene transcription is regulated by calcineurin/NFAT, CaMKII, CREB, EGFR, MAPK, NFκB, Pax6, PI3K/Akt, *Src,* and WNT/β-catenin signaling pathways. In addition, the 3' untranslated region of EAAT2 mRNA can be processed in multiple ways, all of which affect the efficacy of translation and EAAT2 protein synthesis (Kim et al. 2003). Therefore, comparing signaling pathway data with EAAT2 expression data in Alzheimer dementia and control subjects could help find the best correlates in different brain regions and stages of disease progression. For example, EAAT2 is downregulated in brain regions involved in learning and memory such as hippocampus, in contrast to cerebellum, where EAAT2 is upregulated (Jacob et al. 2007). Recent work in positron emission tomography (PET) imaging of EAAT2 in the brain (Eduardo Zimmer, pers.com) will advance our understanding of the role EAAT2 plays in the development of Alzheimer dementia.

7.12 Lest We Forget

Alois Alzheimer was a psychiatrist and neuropathologist, and a great scientist, born on June 14, 1864 in Marktbreit am Main, Germany (Toodayan 2016). Aged 51, Alzheimer died in December 19, 1915 in Breslau, Silesia (now Wroclaw, Poland), where he had been a Professor of Psychiatry at the University of Breslau since 1912. Alzheimer published two papers in 1907 and 1911 describing "amyloid plaques and neurofibrillary tangles" in the brain autopsies of his two presenile patients, Auguste Deter and Johann F. (Alzheimer 1907, 1911). Alzheimer always acknowledged his colleagues, notably Fischer, Redlich and Simchowics, who had described similar structures in the brains of their senile patients (Alzheimer 1911, p. 72). Alzheimer never suggested plaques and tangles were the cause of dementia. Indeed, this is what he wrote in 1911: "So scheint wirklich kein stichhaltiger Grund vorhanden, diese Fälle als durch einen besonderen Krankheitzprozeβ verursacht zu betrachten" (Alzheimer 1911, p. 378). "There is then no tenable reason to consider these cases as caused by a specific disease process" (Förstl and Levy 1991, p. 93). Most interestingly, the re-discovery of Alzheimer's records and neuropathology slides of his two patients, by Graeber and colleagues in 1995 and 1997, made it possible to determine that Auguste Deter had carried the PS1 mutation F176L (Graeber 1999; Müller et al. 2013).

Acknowledgements I have no conflict of interest, actual or potential. I thank Hans-Jürgen Apell, Anton Scott Goustin, Anna Seelig, Anna Thuring, Jack de la Torre and David Woolls for comments and interest, and Risto Kurkinen for help with the Figures. Special thanks to Manuel Graeber for interesting comments, and the two Alzheimer's papers, as well as showing me the plaques and tangles in the original brain slides of Auguste Deter and Johann F.

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Chapter 8
The Glutamatergic System as a Target for the Development of New Pharmacological Treatments of Bipolar Disorder

Grigorios N. Karakatsoulis and Konstantinos N. Fountoulakis

Abstract Bipolar affective disorder is a relatively common psychiatric disorder that affects millions of people worldwide. Existing antidepressants and mood stabilizers used to treat it are not always sufficient for all patients and typically depression in patients with bipolar disorder (BD) is not well responding to antidepressants. Glutamate is the major excitatory neurotransmitter of the CNS and is present in more than 50% of nerve tissue. The relationship between glutamatergic dysfunction and the pathophysiology of depression either unipolar or bipolar has been documented over the past 20 years. Although the first clinical trial investigating ketamine's potential antidepressant effect was conducted approximately 25 years ago, and the relevant literature is relatively limited, new pharmaceutical agents targeting the glutamatergic system are under investigation. The glutamate hypothesis of etiology of mood disorders is expected to complement and improve the prevailing monoamine hypothesis and may indicate novel therapeutic targets. There are currently few pharmacological agents that act on the glutamatergic system and have been approved for the treatment of bipolar disorder. In fact, only S-Ketamine has been recently approved (2019) in the United States FDA from March 2019. Current research is Focused on: a) broad glutamatergic modulators such as ketamine, S-ketamine, dextromethorphan, AVP-786, nitric oxide (N2O), AZD6765, b) *N*-methyl-D-aspartate (NMDA) specific subunit receptor antagonists (NR2B) such as CP-101,606, MK-0657 (CERC-301), c) some NMDA receptor glycine site agonists such as D-cycloserine (Chen et al. 2019), GLYX-13, sarcosine, AV-101,d) d) metabotropic glutamate receptors (mGluRs) modulators such as AZD2066, RO4917523/basimglurant, JNJ40411813/ADX71149, R04995819). There are even other potentially interesting targets of the glutamate receptor with preclinical antidepressant-like efficacy, including AMPA agonists (for example, CX-691/ORG 2448 and ORG-26576) as well as mGluR agonists (Fountoulakis et al. 2012a, b). The range of spectrum of the above recent findings dictates most intensive and targeted study based on both preclinical and clinical studies of agents that influence

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_8

the glutamatergic system with the aim of developing new effective therapies for bipolar disorder.

Keywords Bipolar disorder · Glutamate · Novel · Treatments · Molecular components

8.1 Historical Perspective Introduction for Bipolar Disorder

Bipolar affective disorder is a relatively common, chronic, and recurrent psychiatric disorder that affects millions of people worldwide. Existing antidepressants and mood stabilizers used to treat it are not always sufficient for all patients and typically depression in patients with bipolar disorder (BD) does not respond well to antidepressants. These patients have delayed onset of antidepressant activity, residual symptoms, low rates of remission, and more frequent relapses. Consequently, the need for developing targeted antidepressants in patients with treatment-resistant bipolar disorder is pressing.

Over the past 20 years, the relationship between glutamatergic dysfunction and the pathophysiology of depression has been documented, and it has been shown that glutamatergic activity has multiple goals for the development of new antidepressants. NMDA antagonists have been shown to produce rapid antidepressant effects in various preclinical models (Zarate et al. 2012).

For at least 75 years glutamic acid has been known to affect the central nervous system (CNS) but its role as a neurotransmitter was not recognized until 1984 (Fonnum 1984). Glutamic acid is the major excitatory neurotransmitter in the mammalian CNS. The initial interest in the contribution of the glutamatergic system to the pathophysiology of neuropsychiatric diseases was born after the discovery of ischemic-mediated glutamatergic neurotoxicity following a stroke.

Following this finding, an increasing number of studies on glutamatergic signaling in the affected brain have been observed. Current research is focused on glutamatergic neurotransmission, which is considered a suitable therapeutic target for both mental disorders such as major depressive disorder, bipolar disorder, schizophrenia, mental disorders such as cardiovascular and cardiovascular disorders. multiple sclerosis or motor neuron disease (ALS). Apart from the glutamatergic system, the cholinergic system (e.g., scopolamine), the endogenous opioid system (e.g., factor ALKS-5461), antagonists of the corti-cotrophin releasing factor receptor (e.g. OPP, CP-316,311) are concidered as potential therapeutic targets that promote rapid antidepressant activity (Skolnick et al. 2009, Fountoulakis et al. 2012b).

The involvement of glutamatergic dysfunction with mood disorders was initially based on preclinical data related to NMDA antagonists. Postmortem studies of patients with BD revealed reduced expression of both the NR1 subunit of NMDA receptors in the frontal cortex and several subunits of the same receptor as well as

AMPA and kainate receptors in the midbrain (Hashimoto et al. 2007). Other similar studies have also shown reduced expression of metabotropic glutamate receptors (mGluRs) in the frontal cortex of patients with bipolar disorder. Parallel genome-wide association studies (GWAS) have revealed genetic evidence that glutamate signaling is involved in the pathophysiology of bipolar disorder.

Subsequently, neuroimaging studies in patients with bipolar disorder confirmed the above findings (Li et al. 2018). A meta-analysis of studies of 1H-MRS (Proton magnetic resonance spectroscopy) from 1980 to 2010 on the levels of glutamic acid in the brains of patients with bipolar disorder showed extensive elevation of *Glx* in the frontal cortex compared with healthy controls. The same result was confirmed and in subsequent meta-analyses, however it was further found that many factors including patient disposition and medication may affect glutamine levels. Of the specific pharmacological agents that have action on glutamic acid receptors, the first to be found to have therapeutic efficacy in bipolar disorder and major depressive disorder is ketamine.

Ketamine was discovered in 1962, first tested in humans in 1964, approved for use in the United States in 1970, and is currently used clinically for anesthesia for brief surgical procedures. Its composition was derived from phencyclidine and cyclohexamine and was initially used for its anesthetic and analgesic properties presenting hallucinations and dissociative symptoms (mainly amnesia) as adverse effects from the mental sphere. It is also used to treat postoperative pain, chronic cancer and neuropathic pain, as well as a sedative in the emergency department. More recently it has been shown to have antidepressant properties in both major depressive disorder (MDD) and bipolar depression (Grady et al. 2017).

The antidepressant properties of ketamine were first found in small studies in 2000 and 2006, though the first clinical trial investigating its potential antidepressant effect was conducted approximately 25 years ago (Diazgranados et al. 2010).

Ketamine is an *N*-methyl-D-aspartate receptor antagonist (NMDAR). It is a racemic mixture comprising equal portions of (R)-Ketamine and (S)-ketamine, producing rapid and prolonged antidepressant activity in treatment-resistant patients with major depressive disorder (MDD) or bipolar depression (BD) (Zarate et al. 2006) and presents some important advantages such as rapid antidepressant effect (Fountoulakis and Vieta 2008, Fountoulakis et al. 2007) and the absence of some adverse effects of the SSRIs such as sexual dysfunction and body weight changes. It may also increase the efficacy of electroconvulsive therapy (ECT) and may be preferable for anesthesia in the surgical operations of patients with depression.

Its major disadvantages are that its therapeutic effects are temporary, weakening after days to weeks (with response rates above 60% 4.5 h after a single dose, and approximately 40% after 7 days), although more are reported long-term effects in some patients and there is always the risk of tolerance and dependence (Caddy et al. 2014). Oral ketamine has limited bioavailability (17% to 20%). Therefore, the main route of administration of ketamine is intravenous (IV).

Ketamine is water soluble and has a short half-life (1–3 h) and is metabolized to dehydro-ortamine, norketetamine, and hydroxynoramethamine. Of these metabolites, norketetamine is active and accounts for one third of the analgesic potency of

ketamine. The liver enzymes that biotransform ketamine are CYP3A4, 2B6, and 2C9.

Despite its clear antidepressant efficacy, ketamine has been associated with significant long-term side effects including: psychotic and withdrawal symptoms, cognitive deficits (such as severe working memory impairment, episodic and semantic memory), urotoxicity, dangerous fluctuations in heart rate and blood pressure, visual acuity, drowsiness (Grady et al. 2017; Short et al. 2018), and the possibility of abuse as previously mentioned (Li et al. 2011).

Because (R)-ketamine has a lower affinity for NMDA than the (S)-ketamine enantiomer (which exhibits approximately four times more affinity for the NMDA glutamate receptor in vitro than R-ketamine) (Molero et al. 2018) but mainly because of its serious side effects it has not been approved for use as an antidepressant in contrast to (S)-ketamine (Dhir 2017; Fountoulakis et al. 2017; Hashimoto 2019). Developed as a nasal spray formulation and approved in the United States Food and Drug Administration (FDA) from March 2019, as a drug against to treatment-resistant depression (TRD). In recent years, in addition to S-ketamine, new effective therapeutic agents for bipolar disorder with properties of glutamatergic modulators have been studied. Such factors are: (a) broad glutamatergic modulators such as dextromethorphan, dextromethorphan-quinidine combination, nitrogen dioxide (N2O), factor AVP-786 and AZD6765, traxopropyl-C65; 301), (b) glycine site agonists such as D-cycloserine, sarcosine, the GLYX-13 and AV-101 factor and their regulators, (c) metabotropic glutamate receptors such as AZO2066, RO4917523/basimglurant, JNJ40411813/ADX71149, R04995819 (RG1578) (Henter et al. 2018).

In conclusion the knowledge that glutamatergic dysfunction plays a critical role in the pathophysiology of bipolar depression has paved the way for the study of multiple molecular targets of glutamatergic dysfunction in order to develop more effective and safer antidepressants with new mechanisms of action. This path is just beginning and requires a great deal of research. In any case, the optimism is justified that the glutamatergic system is the main area of development for the next generation of therapeutic agents for bipolar disorder and many other psychiatric disorders.

8.2 The Glutamatergic System in Bipolar Disorder

As the main stimulus neurotransmitter in the central nervous system, glutamate, as measured in combination with glutamine (Glx), is involved in many psychopathological conditions when its levels are disturbed. Bipolar disorder (BD) is one of these conditions as elevated levels of Glx are observed in the sufferers (Smaragdi et al. 2019; Dalvie et al. 2016).

Bipolar disorder (BD) is also strongly associated with glutamate/GABA-glutamine cycle disorders. This has also been demonstrated by magnetic resonance spectroscopy (MRS) studies that confirm elevated levels of glutamate (Glu) as well

as the sum of glutamate and glutamine (Glx) in individuals with BD (Soeiro-de-Souza et al. 2015; Yuksel and Ongur 2010).

The hippocampus, thalamus, and glutamatergic neurotransmission pathways associated with these structures are also involved in the pathophysiology of bipolar disorder (McCullumsmith et al. 2007). Chemical neuroimaging studies also confirm the involvement in its pathophysiology of glutamate-dependent neurotransmitter abnormalities in the hippocampus. The same studies also show the highly increased neurotransmitter cell metabolism in the hippocampal neurons of these patients. In addition, abnormalities of the ionotropic glutamate receptors have been identified within their hippocampus although there are still insufficient studies to confirm a similar effect on the metabotropic receptors. In recent years, however, there has been increasing interest in investigating the role of metabotropic glutamate receptors (mGluRs) in BD and their selection as a relevant therapeutic target (Blacker et al. 2017). There are relatively few data available for the study of glutamatergic neurotransmission in the thalamus of patients with bipolar disorder, and these have been derived mainly from a few immune-histochemical studies. These data also indicate glutamatergic neurotransmission disorders at the level of intracellular signaling processes (Ng et al. 2009; Chitty et al. 2015).

In addition to the findings of immune-histochemical studies and chemical neuroimaging, there are currently two theories regarding the underlying etiology of mood disorders (and therefore bipolar disorder), which include, besides abnormalities in glutamatergic neurotransmission, the over-activation of inflammatory pathways (King et al. 2019).

Thus, it seems that dysfunction of a wide range of monoaminergic, glutamatergic, and immune systems is involved in a complex way in the pathophysiology of mood disorders. One possible point of convergence of these three systems is the kynurenine (KYN) pathway. Ketamine has been shown to affect the essential elements of this metabolic pathway too (Kadriu et al. 2019). Several genes in the glutamatergic system have been found to be involved in the etiology of bipolar disorder. SRC family tyrosine kinase FYN appears to play a key role in the production of anti-NMDAR antibodies in the brain during episodes of BD (particularly in early onset type I BD) (Szczepankiewicz 2009). Conversely, long-term lithium treatment is found to induce lower levels of anti-NMDAR antibodies, fact which may explain the lithium's stabilization properties (Ferensztajn-Rochowiak et al. 2019).

Bipolar disorder and suicidality have been the subject of a genome-wide association study (GWAS). In this study suicide attempt has been evaluated as an independent factor in subjects suffering from bipolar disorder (Willour et al. 2012). Its results have shown strong glutamatergic neurotransmission engagement to the vulnerability of committing suicide (Gaynor et al. 2016).

From the above it can be concluded that the etiology of bipolar disorder is multifactorial. It has multiple dimensions, one of which consists of the glutamatergic system. The evidence appears to be well documented and based on preclinical, clinical, neuroimaging, immune-histochemical, and genomic studies. Consequently,

the growing interest in the development of pharmacological therapies whose action is aimed at the glutamatergic system is justified.

8.3 Molecular Components of the Glutamate Neurotransmitter System as Potential Drug Targets for Bipolar Disorder

Many glutamate regulatory factors have been studied in various preclinical models of mood disorders, and apparently pharmaceutical agents targeting glutamatergic signaling are important new therapeutic approaches to mood disorders.

8.3.1 Glutamatergic Receptors

Glutamate is the major excitatory neurotransmitter of the CNS and is present in more than 50% of nerve tissue. It is also involved in the synthesis of GABA (-γ-aminobutyric acid) (Petroff 2002), which is the major inhibitory neurotransmitter of the mammalian central nervous system.

It is produced from α-ketoglutaric acid, which is an intermediate in the Krebs cycle and is packaged into secretory vesicles in the presynaptic neuron by specific glutamate transporters (Machado-Vieira et al. 2012). It activates several receptors that distinguish their pharmacological and physiological properties in three classes of ionotropic receptors: NMDA, AMPA, and kainate receptors (CA) and in three groups of metabotropic receptors. Twenty-seven genes have been identified for specific subunits of these receptors and a further five proteins are likely to function as receptor-binding proteins or as receptor subunits (Table 8.1).

Table 8.1 The glutamate receptors (Stahl 2020)

Glutamate receptors					
Ionotropic			Metabotropic		
NDMARs	AMPARs	Kainate receptors	*Group I*	*Group II*	*Group III*
			mGluR1/ mGluR5	mGluR2/ mGluR3	mGluR4
					mGluR6
					mGluR7
					mGluR8

8.3.2 *Ionotropic Glutamate Receptors*

Glutamate controls synaptic stimulation in most brain circuits, including the interlimbic pathway but also other pathways involved in synaptic plasticity, learning, memory, behavior (Collingridge and Bliss 1995), and mood disorders.

Several pathophysiological findings have been associated with glutamatergic neurotransmission in persons with mood disorders (Machado-Vieira et al. 2012). Specifically in patients with bipolar disorder, changes in plasma glutamate, serum, and cerebrospinal fluid (CSF) levels as well as elevated levels of glutamate in different areas of the brain have been observed in both live and postmortem studies.

Various types of glutamate ionotropic receptors and their corresponding subunits (NMDA with subunits: NR1C, NR2D, NR3C, NR2D, NR3A and NR3B, AMPA: GluR1, GluR2, GluRu, GluR7, GluR7, GluR7, GluR3, GluR3 have been identified. Apart from them, eight more types of metabotropic receptors (mGluR) associated with G-protein have also been identified and divided into three groups based on the signaling pathway that each one induces: group I (mGlu1 (a, b, c, d) and mGlu5 (a, b) group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7 and mGlu8) (Machado-Vieira et al. 2012). Glutamine binding sites in areas of the brain involved in regulating mood are mainly expressed in the NR2A and NR2B subunits (Magnusson et al. 2002).

Both ionotropic and metabotropic glutamate receptors bind to various intracellular messengers, such as cyclic AMP, Ca2 +, reactive oxygen forms and induce the onset of multiple signaling cascades that determine neuronal growth and development.

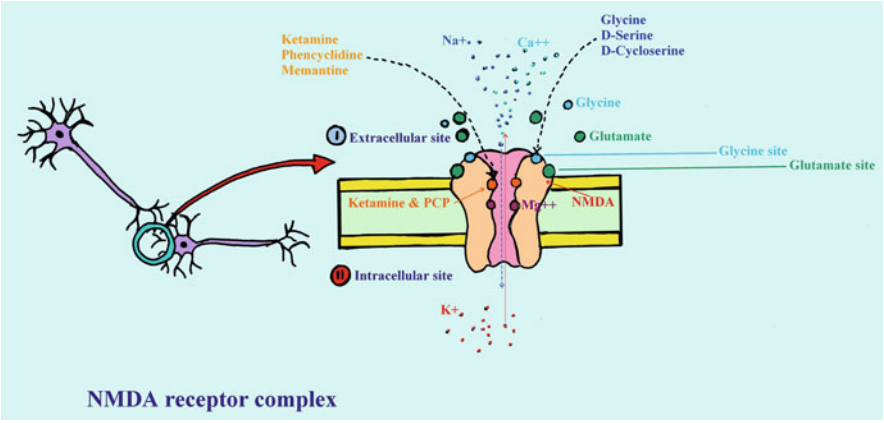

Fig. 8.1 The NMDA receptor complex

8.3.3 NMDA Receptors (NMDARs)

NMDA receptors (NMDARs) are involved in the pathophysiology of several CNS disorders including bipolar disorder (Fountoulakis 2012). A number of their subtypes have been identified, which are formed by alteration of the combination of seven subunits (GluN1, GluN2A-D and GluN3A-B) in quadruple complexes (Fig. 8.1).

These NMDA receptor subtypes exhibit unique structural characteristics, which explain their specific functional and pharmacological properties (Hansen et al. 2018).

Glutamate binds to the agonist site at NMDA receptors. PCP, ketamine, and dizocilpine bind to the PCP receptor in the internal side of the NMDA receptors. Glycine, D-serine, and D-cycloserine bind to a glycine site of the receptor (Kantrowitz et al. 2015). Activation of the NMDA receptor by binding to both NMDA and glycine results in the opening of the channel (Jiang and Amara 2011). This allows voltage-dependent Na^+ and small amounts of Ca^{2+} ions flux inside the cell and K^+ outside the cell.

8.3.4 AMPA Receptors (AMPARs)

AMPA receptors (AMPARs) are activated in the presence of glutamate, causing a rapid stimulation signal. They have a lower affinity for glutamate than NMDA receptors, which allows a faster disintegration of glutamate and therefore rapid inactivation of the receptor (Machado-Vieira et al. 2012) (Fig. 8.2).

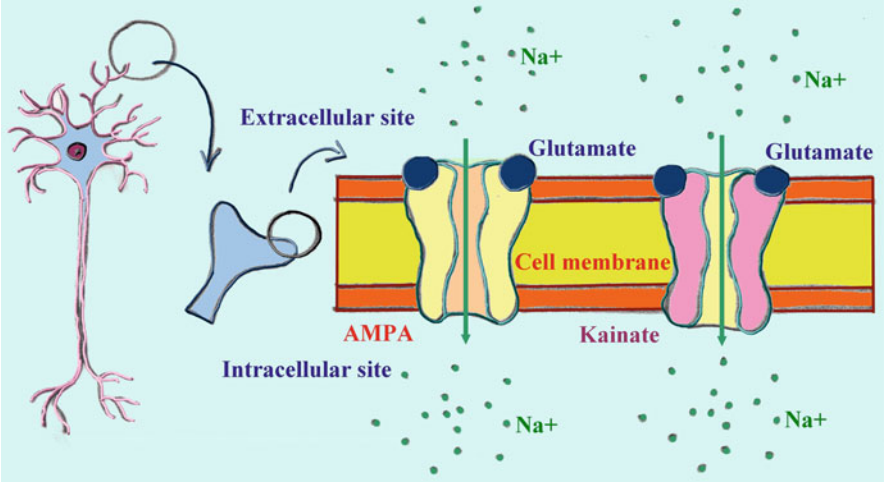

Fig. 8.2 AMPA and kainate receptors

8.3.5 Kainate Receptors (KARs)

Kainate receptors (KARs) have a limited distribution in the brain, and they are believed to affect synaptic signaling and plasticity less than AMPA receptors. KARs present a dual role in stimulating neurotransmission both by activating postsynaptic receptors and by inhibiting γ-aminobutyric acid (GABA) release.

8.3.6 Metabotropic Glutamate Receptors

Group I metabotropic receptors (mGluRs) bind to the phospholipase C signal transduction pathway and localize to both the presynaptic and postsynaptic membrane. Group II and III mGluRs are inhibitory to the adenylyl cyclase pathway and are involved in regulating the release of both glutamate and GABA. Glutamate release is limited by activation of the mGlu2 and 3 presynaptic receptors.

8.3.7 Subunits of the Glutamate Receptors

The first report of glutamate receptor subunit cloning (Hollmann et al. 1989) was published in December 1989, and the beginning of the 1990s marked the beginning of a long series of analogous cloning of such different subunits. The number of these clones was large, and the nomenclature of the various subunits was neither systematic nor common. Thus, different laboratories gave different names for the same glutamate receptor subunit copy, resulting in confusion in the literature. This confusion has been slowly restored and since 2009 the nomenclature of the glutamate receptor subunits has been systematically revised. The nomenclature of the International Union of Basic and Clinical Pharmacology has replaced the previously common names.

8.3.8 Glutamate and Glx Metabolites

Specific imaging studies have also found elevated levels of glutamate and its metabolites in the occipital cortex (OCC) of individuals with BPD (as in individuals with MDD) and decreased levels in the anterior cingulate cortex (ACC) of these patients (Fountoulakis et al. 2008). Specific magnetic resonance spectroscopy (MRS) studies performed to date show a consistent pattern of elevated levels of Glx (glutamate + glutamine) in subjects with bipolar disorder regardless of mood polarity and reduced levels of Glx and GABA in patients with MDD (Machado-Vieira et al. 2012).

8.3.9 Postsynaptic Density PSD Proteins

Other potential therapeutic targets of bipolar disorder that include glutamatergic dysfunction include postsynaptic density proteins (PSD) (Machado-Vieira et al. 2012). These are proteins such as PSD95, SAP102, and others, which interact with ionotropic glutamate receptors in the synaptic membrane and modulate signal transduction. Reduced levels of PSD95 have been observed in the toothed helix of individuals with bipolar disorder. SAP102 protein has been shown to decrease NMDAR-2B subunit expression in individuals with mood disorders.

8.3.10 Excitatory Amino Acid Transporters (EAATs)

The major role of the Excitatory Amino Acid Transporters (EAATs) is to clear glutamate from the extracellular space (these are transmembrane proteins that utilize the electrochemical gradient to slowly transfer glutamic acid from the extracellular space into the glial cells).

In postmortem studies of people with affective disorders, decreased expression of various EAATs has been observed. On the contrary, it has been observed that β-lactam antibiotics induce increased expression of these transporters. Mood stabilizers, such as valproate and lamotrigine, are also inducers of EAAT activity through a different pharmacological mechanism. Conversely, in various preclinical models, EAAT competition has been shown to have depressive symptoms. EAATs have also been shown to mediate synaptic transmission, as well as their function serves the recycling of the neurotransmitter and protects the neurons from the toxicity of its excess (Zerangue and Kavanaugh 1996).

8.3.11 Vesicular Glutamate Transporters

Significantly reduced expression of VGLUT1 mRNA in the entorhinal cortex (ERC) of patients with both bipolar disorder and major depressive disorder has been found (Bai et al. 2001). It has also been found that various antidepressants increase the expression of VGLUT in the limbic system and the same has been observed after lithium treatment. This latter observation sheds light on a possible mechanism that explains the protective action of lithium against glutamate-induced stimulatory toxicity.

In conclusion it is therefore possible to develop antidepressants, not only by modulating the ionotropic and metabotropic receptors, but also by altering synaptic glutamate concentrations by developing appropriate agents that will affect receptor subunits and neurotransmitter metabolites, in postsynaptic density proteins (PSD)

and excitatory amino acid transporters (Excitatory Amino Acid Transporters, EAATs).

8.4 Current Treatments and Modulators of Glutamatergic Signaling for Bipolar Disorder

Currently there are few pharmacological agents that act on the glutamatergic system and have indication for the treatment of bipolar disorder. Only S-ketamine has been recently approved (2019) in the United States FDA from March 2019 and developed as a nasal spray formulation, although not indicated for bipolar disorder but as a drug against treatment-resistant depression (TRD). Other medications with action on glutamatergic system which are currently used for the treatment of BD are *lamotrigine* and *memantine* although only the first has official indication. Current research is investigating other agents such as riluzole and (R)-ketamine (PCN-101, HR-071603), whose efficacy in the treatment of bipolar disorder is expected to be established.

8.4.1 Ketamine

Ketamine has been studied in the last decade for its efficacy in bipolar depression as well as for major depressive disorder (Singh et al. 2017). Its antidepressant properties are believed to be due to the competition of *N*-methyl-D-aspartate receptors (Grady et al. 2017). Oral administration of ketamine is not particularly effective. Sublingual administration has a better bioavailability of ~30% and a lower rate of conversion to *norketamine* compared to oral administration. Intravenous administration of ketamine at a dose of 0.5 mg/kg produces rapid and potent antidepressant effects (Thomas et al. 2018); however, such administration is not practical. In addition to its beneficial effects, it is a potentially addictive drug. At the same time, its prolonged use has been associated with a decline in cognitive functions and alterations that are apparent in imaging brain processes. Current data suggest that a single intravenous ketamine infusion is effective for patients with bipolar depression without psychotic symptoms who have no comorbidity with substance dependence (Grady et al. 2017; Lapidus et al. 2013).

8.4.2 S-ketamine

S (+)-ketamine is the pure dextrorotatory enantiomer of ketamine and has been in use in anesthesiology approximately for the last 25 years. Its main action is due to

non-competitive inhibition of the NMDA receptor while simultaneously is acting on monoamine, opioid, adenosine, and other purinergic receptors (Trimmel et al. 2018). S-ketamine has antidepressant effects similar to ketamine. At the same time it seems to be better tolerated with less psychotomimetic side effects than racemic ketamine (Paul et al. 2009). U.S. Food and Drug Administration approved esketamine in nasal spray in March 2019 in combination with oral antidepressant medication to treat adults suffering from treatment-resistant depression. However, it has not yet received formal approval for the treatment of bipolar depression.

8.4.3 Lamotrigine

Lamotrigine has been in use as an anticonvulsant since 1991 and has been approved as a maintenance treatment for bipolar disorder II in June 2003 (as the first drug of this class after lithium). It has proven its efficacy mainly in the relapse prevention of bipolar depression. Pharmacologically it is inhibitor of glutamate release while simultaneously is increasing the expression of the AMPA receptor through inhibition of the voltage-dependent channels of sodium, potassium, and calcium. It has also neuroprotective action, slightly inhibits serotonin reuptake and has activity of an agonist of gamma aminobutyric acid (GABA). It is less effective in treatment-resistant bipolar depression and rapid cycling disorder. It requires relatively long time for starting its action.

8.4.4 Memantine

Memantine is an NMDA receptor inhibitor that has been in clinical use since 1982 and has been approved for Alzheimer's dementia. It is a low affinity voltage-dependent uncompetitive antagonist of NMDA glutamatergic receptors. Memantine inhibits the prolonged intracellular influx of Ca2 + ions binding primarily to extracellular NMDA receptors (Parsons et al. 2007). At the same time, it prevents desensitization of dopaminergic receptors, caused by antidepressants, which may induce further depressive symptoms. It is considered effective in the treatment of acute mania and in the prevention of both manic/hypomanic and depressive relapses of bipolar disorder. Although it has no official indication, it can be used to treat bipolar disorder that does not respond to classic mood stabilizers (Serra et al. 2014). Common side effects include drowsiness, dizziness, headache, and constipation. However, it can also have serious side effects such as heart failure, thrombosis, and psychotic symptoms.

8.5 Emerging Treatments Related to Glutamate Modulating Drugs for Bipolar Disorder

Both preclinical and clinical studies have implicated glutamatergic dysfunction in the pathophysiology of mood disorders, such as bipolar depression and major depressive disorder (MDD). In particular, rapid reductions in depressive symptoms were observed in response to hypoanesthetic doses of ketamine in subjects with bipolar depression or MDD. These results have led to the development of other glutamatergic modulators for use either as monotherapy or as adjunctive therapy.

The antidepressant effects on bipolar depression of various glutamatergic modulators are investigated, including: (1) broad glutamatergic modulators (ketamine, S-ketamine, dextromethorphan, AVP-923, AVP-786, nitric oxide (N_2O), AZD6765). (2) *N*-methyl-D-aspartate (NMDA) specific subunit receptor antagonists (NR2B) (CP-101,606, MK-0657 (CERC-301)), (3) some glycine site agonists (D-cycloserine, GLYX-13, sarcosine, W-101), and (4) glutamate receptor modulators (AZO2066, RO4917523/basimglurant, JNJ40411813/ADX71149, R04995819).

There are even other potentially interesting targets of the glutamine receptor with preclinical antidepressant-like efficacy, including AMPA agonists (for example, CX-691/ORG 2448 and ORG-26576) as well as mGluR7 agonists.

8.5.1 Wide Glutamatergic Modulators

8.5.1.1 Ketamine

Ketamine is by far the best-studied glutamatergic agent in the treatment of affective disorders, with a very low risk (similar to placebo) of causing hypomania or mania, indicating that its use is safe for people with bipolar depression while at the same time is also known to have anti-suicidal activity. In all studies to date which investigate its efficacy in bipolar depression, it has been used in combination with conventional mood stabilizers such as lithium and valproate.

At the cellular level, increased glutamatergic capacity is responsible for the antidepressant effects of ketamine. It is still possible that increased glutamatergic efficiency of AMPA receptors over NMDA receptors following ketamine administration may enhance the dynamics of neuronal synapse and activate genes which encode neuronal plasticity-mediated proteins. Ketamine has been shown in experiments in rats to rapidly activate the mTOR pathway, leading to an increased synthesis of signaling proteins and an increased number of new synapses in the prefrontal cortex (Henter et al. 2018).

The (S)-ketamine isomer has been found to block NMDA receptors with greater potency than the (R) isomer, but without similar antidepressant effects. It has been found that its antidepressant activity is produced by one of its metabolites, hydroxynorketamine (HNK) via an independent mechanism that appears to increase

AMPA receptor activation. The metabolites of (2S, 6S, 2R, 6R) -HNK are also pharmacologically active.

From its side effects the most severe acute of these are the transient psychotic-like and dissociative episodes, increased blood pressure, and tachycardia while chronic side effects such as drug abuse and dependence, dissociative symptoms (e.g., dissociative amnesia), ulcerative cystitis, and neurotoxicity should be considered. The limitations and the serious side effects of ketamine's use led to the development of more effective relevant pharmacological agents with fewer side effects. Relevant emerging therapeutic approaches to bipolar disorder that target the glutamatergic system will be presented below.

8.5.1.2 R-ketamine (PCN-101, HR-071603)

R-ketamine is the (R)-(-)enantiomer of ketamine. Preclinical studies appear to have a faster onset, stronger and longer-term antidepressant activity than S-ketamine at least in animal models of depression (Zhang et al. 2014). R-ketamine has been undergoing trials for the treatment of depression under the code PCN-101 since November 2019.

It is believed that the better efficacy of both R-ketamine and S-ketamine over racemic ketamine may be due to the strong competition of alpha-7 nicotinic receptors, both by themselves and their metabolites norketamine and hydroxynorketamine (Yang et al. 2015).

8.5.1.3 Dextromethorphan (DXM), AVP-923, AVP-786

Dextromethorphan is a non-selective, non-competitive antagonist of NMDA receptors better known as an antitussive agent. It also has action on opioid receptors and at higher doses it acts as an agonist for the sigma-1 receptor and as an inhibitor of the norepinephrine and serotonin transporters. To date, there is no randomized controlled trial investigating the use of dextromethorphan as a monotherapy for the treatment of mood disorders. The efficacy of the combination of dextromethorphan with quinidine (named *AVP-923*) is currently being studied in treatment-resistant depression at oral dosage of 45 mg dextromethorphan and 10 mg quinidine twice daily. The above combination presents mild side effects from the digestive system, sedation, dizziness, and possibly insomnia. It also inhibits cytochrome 2D6, thereby increasing plasma dextromethorphan levels. The combination of deuterated (d6) -dextromethorphan with an extremely low dose of quinidine *(AVP-786)* as well as deuterium incorporation (^{2}H) optimizes the reduction of first-pass metabolism of both dextromethorphan and quinidine, thereby reducing the risk of cardiological adverse reactions and interactions with other drugs (Henter et al. 2018).

8.5.1.4 Nitrous Oxide (N_2O)

Nitrous oxide (N_2O) is an inhaled general anesthetic most commonly used in dental or short obstetric surgical operations. It is a non-competitive NMDA receptor inhibitor and has a broad mechanism of action similar to that of ketamine. Inhalation of a mixture of 50% N_2O and 50% nitrogen does not cause psychotic-like symptoms and its side effects include anxiety, headache, and nausea/vomiting. To date N_2O is used only in a research setting (Tym and Alexander 2011).

8.5.1.5 Riluzole

Riluzole is a glutamic acid modifier approved for the treatment of ALS. It reduces extra-synaptic glutamate by inhibiting presynaptic release and simultaneously enhances astroglial glutamate uptake. Its antidepressant activity has been demonstrated in animal models, but to date no randomized controlled trials (RCTs) using riluzole have been published in patients with mood disorders. However, there are several case reports describing recession rates of depressive symptomatology from 21 to 32%. In at least one such case study of bipolar depression, increased doses of riluzole at 50–200 mg per 24 h in combination with lithium resulted in significant remission of depressive symptoms during the 5th to 8th week of treatment without developing hypomania or mania.

8.5.1.6 AZD6765

AZD6765 is a non-selective low affinity NMDA receptor channel inhibitor whose development was discontinued in 2013 and is reported here for historical reasons. It was administered intravenously at 50, 100, and 150 mg and showed no psychotic-like symptoms.

8.5.2 Subunit NR2B-Specific NMDA Receptor Antagonists

NR2B is a subunit of the NDMA receptor. Studies of the therapeutic effects of NMDA receptor subunit antagonists were based on the hypothesis that agents that selectively block the NMDA receptor are by definition specific and have fewer undesirable side effects than broad glutamatergic modulators. Two agents of this class have been investigated to date: *CP-101,606* and *MK-0657* (recently renamed *CERC-301*).

8.5.2.1 CP-101,606

CP-101,606 is a selective antagonist of the NR2B subunit of the NMDA receptor which has been used only intravenously at a dose of 0.75 mg/kg per hour for 1.5 h followed by 0.15 mg/kg per hour for the next 6.5 h. The development of this agent stopped due to potentially cardiovascular toxicity (QT c prolongation).

8.5.2.2 MK-0657 (CERC-301)

MK-0657 (CERC-301) is an oral selective NR2B subunit antagonist of the NMDA receptor. In contrast to CP-101,606, no serious adverse reactions were observed during the relevant trials. However, there was no statistically significant improvement in depressive symptoms over placebo (after MADRS evaluation) at oral administration of at least 20 mg, although at the same dosage some improvement was observed using the HAM-D scale, as well as using the BDI self-report scale.

8.5.3 NMDA Receptor Glycine Site Partial Agonists

8.5.3.1 D-Cycloserine (DCS)

D-cycloserine (DCS) is a broad-spectrum antibiotic and at doses greater than 100 mg/24 h, acts as an NMDA receptor glycine site partial agonist. Recent studies evaluating the efficacy of agents acting on the NMDA receptor have found that D-cycloserine exhibits at high dosage (1000 mg) a rapid antidepressant response, although not at a lower dosage (250 mg).

Its side effects include symptoms from the gastrointestinal system, dizziness, tinnitus and vision disorders, sleep disorders, peripheral neuropathy, and even depressive symptoms. Epileptic seizures and psychotic symptoms are also reported in $<5\%$ of the patients who are treated with D-cycloserine (Machado-Vieira et al. 2017; Dang et al. 2014).

8.5.3.2 GLYX-13

GLYX-13 is also an NMDA receptor glycine site partial agonist, administered intravenously at a dosage of 5–10 mg/kg and which has encouraging preliminary results in the treatment of depressive symptomatology. Since early 2016 it has been approved by the FDA as a complementary treatment for major depressive disorder. In contrast to the intravenous injection of NMDA receptor antagonists, the corresponding injection of GLYX-13 at any dose did not cause neither severe adverse reaction (with the exception of dizziness at 10%) nor dissociative symptomatology (Lener et al. 2017; Henter et al. 2018).

8.5.3.3 Sarcosine

Sarcosine is a glycine-I transporter inhibitor and enhancer of NMDA receptor function. To date, its therapeutic potential has not been fully evaluated. It is effective in oral administration at a dosage of 1000–1500 mg/24 h. The most important side effects reported are relatively mild sleep disorders.

8.5.3.4 4-Chlorokynurenine (AV-101)

4-Cl-KYN (AV-101) is a highly selective, potent glycine site antagonist of the NMDA receptor. Its action as a rapid-acting antidepressant is under trial. It is administered orally at a dosage of 1.080 to 1.440 mg/24 h. In 2019 a phase II clinical trial had negative results for major depressive disorder.

8.5.4 Metabotropic Glutamate Receptors (mGluRs)

Metabotropic glutamate receptors (mGluRs), which are observed in both neurons and glial cells, are an additional glutamate signaling pathway in addition to NMDA and AMPA receptors and are found throughout the whole brain.

The hypothesis of the efficacy of mGluR2 agonists in the treatment of depressive symptoms in both major depressive disorder and bipolar disorder is based on their reduction in glutamate release.

By an opposite mechanism, antidepressant effects are expected from mGluR2/3 antagonists, which enhance glutamate levels in the synaptic cleft, thereby enhancing the transmission rate of AMPA receptors by causing increased extracellular monoamine levels. Although several agents (either positive or negative allosteric modulators) are under trial for treatment-resistant depression, at present there is no strong evidence of their antidepressant effect.

8.5.4.1 AZD2066

AZD2066 is an mGluR5 antagonist that exhibits antidepressant properties. It seems to be an ineffective agent in oral administration. Dosage ranges from 12–18 mg/24 h. Side effects include mild gastrointestinal symptoms, mild sleep disorder, and headache.

8.5.4.2 RO491753/BasimglurantRO4917523

Basimglurant, RG7090 is an mGluR5 negative allosteric modulator. It is also not an effective agent in oral administration. Its dosage range fluctuates from 0.5 mg to 1.5 mg/24 h. Adverse reactions include mainly dizziness (23%) and two cases of mania triggering have been reported.

8.5.4.3 JNJ40411813/ADX71149

JNJ-40411813/ADX71149 is an mGluR2 positive allosteric modulator that does not appear to outperform at least its antidepressant effects over other agents in the same class. It is also ineffective orally. Its dosage range fluctuates from 50 to 150 mg. The most common side effect is vertigo.

8.5.4.4 RO4995819

RO4995819 is an mGluR2/mGluR3 antagonist and negative allosteric regulator that has antidepressant efficacy but its development for MDD resistant to treatment has been discontinued. It was also not effective in oral administration and its dosage ranged from 5 to 30 mg/24 h.

8.6 Conclusion and Future Perspectives

Currently available therapeutic agents used to treat bipolar disorder act on multiple molecules and receptors. A fundamental issue for the discovery of innovative therapies is the understanding of the combination of cellular interactions and the molecular mechanisms which are responsible for bipolar disorder.

Recent findings from research into the treatment of bipolar disorder suggest that the effects on NMDA receptors may need to be combined with other cellular and/or molecular effects to provide effective therapeutic responses. Such combinatorial interactions warrant the most intensive and targeted study based on both preclinical and clinical studies of agents that influence the glutamatergic system.

Research to date justifies the optimism that new pharmaceutical agents targeting the glutamatergic system may play an important role in the treatment of bipolar disorder. In the above framework, ionotropic glutamate receptors appear to be predominant, despite the fact that ketamine and other glutamatergic modulators that work with a similar mechanism may not be widely recommended as treatment in clinical practice.

However, ketamine may be considered a therapeutic option for cases of bipolar depression that are resistant to treatment, either for the temporary relief of symptoms or as transitional therapy in view of alternative methods. In contrast, S-ketamine has proven its efficacy clinically through its wide glutamatergic action. Factors belonging to the broad glutamatergic modulators such as N_2O and *AVP-923* are promising; however, more research is needed to establish their efficacy and safety.

A significant number of broad and selective NMDA receptor antagonists that are withdrawn after failure to demonstrate efficacy for other indications are currently under investigation, and if proven to be effective, they may be alternative therapies that deprive ketamine's psychotic-like and dissociative side effects. In addition to these other NMDA receptor antagonists, which have actually demonstrated antidepressant properties, some factors such as GLYX-13 and CERC-301 appear to have encouraging preliminary results. It should be noted, however, that none of these agents at least currently appear to exhibit rapid antidepressant activity, efficacy against durable symptoms, single-dose efficacy as well as anti-manic/anti-suicidal/anti-anhedonic properties and stabilizing action.

The metabotropic glutamate receptor modulators so far do not appear to be particularly effective in clinical trials which are relevant with the development of new therapeutic agents for bipolar disorder. Besides, there are currently no new agents (after lamotrigine) that act on the glutamatergic system and have proven mood stabilizing properties.

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Chapter 9
CNS Glutamate in Impulsive Aggression

Alan R. Felthous and Joe Nassif

Abstract Glutamate appears to be a critical neurotransmitter in the neurocircuitry and neurophysiology of aggressive behaviors in mammals including humans. Animal models help to clarify the neurocircuitry of both defensive and predatory aggression. Together with other neurotransmitters, glutamate is involved in the medial amygdala–mediobasal hypothalamus–dorsal periaqueductal gray pathway which triggers defensive aggression in animal models and impulsive aggression in humans. CSF glutamate levels are shown to be elevated in humans with impulsive aggression. The growing knowledge about glutamate's potential role in abnormal impulsive aggression may help to explain antiaggressive mechanisms of action of anti-impulsive aggression and antipsychotic agents as well as memantine, an N-methyl-D- aspartate antagonist, through restoration of a glutamate/GABA imbalance.

Keywords Anti-impulsive aggression agents · Glutamate · Glutamate/GABA balance · Impulsive aggression · Neurocircuitry of aggression · Top-down/bottom-up hypothesis

9.1 Introduction

According to Barratt's tripartite classification of human aggression (Barratt 1991), medical or secondary aggression typically improves from pharmacotherapy of the primary disorder such as bipolar disorder with aggression associated mania; impulsive aggression often responds to an anti-impulsive aggression agent (AIAA) (Felthous and Stanford 2015; Lee et al. 2019), whereas premeditated, nonpsychopathological aggression has not been shown to improve with any pharmaceutical agent (Felthous and Barratt 2003).

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_9

A longstanding and continuing flaw in research on the pharmacotherapy of aggression has been the failure to identify the type of aggression that is being treated (Barratt and Slaughter 1998; Felthous et al. 2013), and to distinguish impulsive aggression in particular (Felthous and Stanford 2015). Where the literature on the pharmacotherapy of aggression does not specify the type of aggression or leaves the definitions broad or vague, results of drug trials have been and will continue to be inconclusive. For example, the review by Huband and colleagues on antiepileptic therapy for aggression did not limit the focus to primary impulsive aggression and broadened the study population to include various psychopathological conditions (Huband et al. 2014): results were unsurprisingly mixed. Where reviews and studies focus on primary impulsive aggression, aggression that is not only recurrent but defined and diagnosed as impulsive, the results are much more consistent (Felthous et al. 2013).

9.2 The Neurocircuitry of Impulsive Aggression

9.2.1 Animal Models and the Neurocircuits of Impulsive and Predatory Aggression

Animal models support two neuropathways for two distinctly different types of aggression, manifested somewhat differently in different species. Subsequently two separate but closely located neurocircuits have been identified in the cat for defensive/affective (impulsive) aggressive behavior and quiet biting attack/biting aggression (predatory or premeditated) behavior. For defensive/affective behavior the ascending pathway has been traced using radioautography to the antero-medial hypothalamus and the medial thalamus. Descending projections pass through the central tegmental areas of the midbrain and pons, locus coeruleus and motor and sensory nuclei of the trigeminal complex (Shaikh et al. 1987). Activation of the medial amygdala-mediobasal hypothalamus-dorsal periaqueductal gray pathway triggers hyper-arousal-driven aggression (Toth et al. 2012), i.e., defensive rage, comparable to reactive or impulsive aggression in humans.

When Hess elicited defensive aggression in the cat by electrical stimulation of the hypothalamus (Hess 1927), he provided the first evidence for its involvement. Subsequent demonstration that injection of L-glutamate into the hypothalamus also elicited the aggressive reaction (Brody et al. 1969) suggested that this neurotransmitter may be involved in hypothalamic aggression. Glutamate projections act on the N-methyl-D-aspartate (NMDA) receptors in the dorsolateral periaqueductal gray. Demonstrating evolutionary conservation, the aggressive response from stimulating the hypothalamus has been shown in various animal species ranging from fish and lizards to monkeys and humans (Haller 2013).

Hypoarousal-related aggression is associated with activation of the central amygdala-lateral hypothalamus-ventral periaqueductal gray pathway (Tulogdi

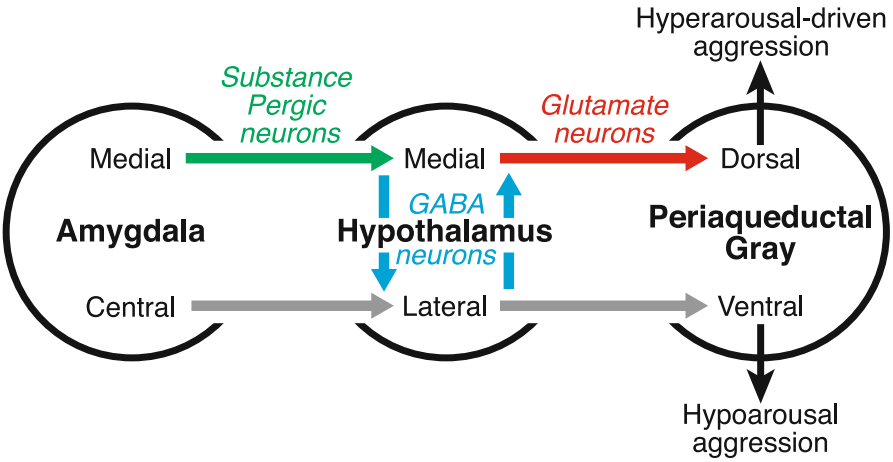

Fig. 9.1 The two parallel tracks for hyperarousal and hypoarousal aggression can inhibit each other with bidirectional GABA neurons in the hypothalamus

et al. 2010). This type of aggression is known as predatory (Haller 2013), and may be analogous to proactive, predatory, or premeditated aggression of humans. The periaqueductal gray (PAG) is involved in aggression-induced autonomic responses (Bandler and Shipley 1994; Behbehani 1995) with the dorsolateral PAG increasing the heart rate and associated with affective hyperexcitatory aggression whereas activation of the ventrolateral periaqueductal gray is associated with hypoexcitatory, predatory aggression (Tulogdi et al. 2010).

Hypothalamic mechanisms of aggression are influenced by neural connections with multiple neurotransmitters including acetylcholine, dopamine, GABA, glutamate, noradrenaline, serotonin, substance P, and vasopressin (Haller 2013). Dopaminergic and noradrenergic inputs as well as substance Pergic neurons from the medial amygdala activate the mediobasal hypothalamus in cats, which project to the periaqueductal gray via glutamergic neurons (Siegel et al. 1999, 2007). Through GABAergic projections, the mediobasal hypothalamus inhibits the lateral hypothalamus from mediating predatory aggression (Haller 2013). Conversely the lateral hypothalamus, also via GABAergic neural input, inhibits the mediobasal hypothalamus when predatory aggression is initiated (See Fig. 9.1).

In the rat electrical stimulation of the hypothalamic attack area not only projects to lower brain levels, stimulation also projects upstream to the mediodorsal thalamic nucleus, the piriform and cingular cortices. This allows execution of conspecific attack by enabling location and approach of the opposing rat and overcoming the object's resistance (Halasz et al. 2002).

9.2.2 Neuroimaging of the Neurocircuit of Impulsive Aggression in Humans

The only DSM disorder that is essentially impulsive aggression is Intermittent Explosive Disorder (IED, DSM-5, American Psychiatric Association 2013). Keedy and colleagues recently reviewed neuroimaging studies of subjects diagnosed with IED, wherein some subjects may have had a personality disorder such as borderline personality disorder (Keedy et al. 2019). Studies focused on the orbitofrontal cortex and the amygdala regions which, together with the hippocampus, the corpus callosum, the superior temporal regions, have been associated with aggressive behavior and psychopathic disorders in general (Müller 2007, 2021; Yang and Raine 2007, 2021.

A neuroimaging study showed that subjects with IED had reduced OFC (orbitofrontal cortex) volume (Coccaro et al. 2016), and reduced activations when presented with angry expressions (Coccaro et al. 2007) as well as alteration in the connectivity between the OFC and the right amygdala. Keedy et al. point out that IED subjects had reduced functioning in the frontal brain areas assumed to inhibit and modulate activity in the limbic system (Keedy et al. 2019). Using MRI scans on same-sexed twins, Coccaro et al. found lifetime history of aggression to have modest inverse associations with medial prefrontal ($p < 0.001$) and lateral prefrontal ($p < 0.001$) cortices with significant heritability determined by biometric twin analyses (Coccaro et al. 2018). IED subjects studied with diffusion tensor imaging (DTI) showed reduced white matter integrity in the superior longitudinal fasciculus, a white matter track that connects frontal with temporoparietal regions (Coccaro and McCloskey 2019; Lee et al. 2016).

Neuroimaging studies of IED subjects show reduced gray matter in the right amygdala and the less gray matter, the more extensive the subjects' history of aggressive behaviors. The right amygdala has an inward deformity in IED subjects in comparison with normal controls (Coccaro et al. 2015a). IED subjects have a greater amygdala response to images of angry faces when studied with fMRI (Coccaro et al. 2007). This response is greater in subjects with a more pronounced history of aggressive behavior, with similar findings confirmed by McClosky et al. (2016).

9.2.3 Human Lesion Models in Human Neurocircuits of Impulsive and Premeditated Aggression

In humans stimulation of the posteromedial hypothalamus induces aggression (Bejjani et al. 2002), whereas lesioning this region reduces or eliminates aggressive behavior in violent patients (Sano et al. 1970). Intractable aggression has subsided with continuous electrical stimulation of the "triangle of Sano" (Franzini et al. 2008;

Hernando et al. 2008; Savard et al. 2003). Aggression subsided only gradually and after prolonged intermittent stimulation, but recurred once stimulation was stopped.

Patients with a hypothalamic hamartoma with increased aggression, either affective (impulsive) or predatory (premeditated), show diminution in aggression once the hamartoma is removed (de Almeida et al. 2008; Weissenberger et al. 2001). Hypothalamic hamartomas provide additional evidence for glutamate contributing to aggression in humans. These congenital malformations of the ventral hypothalamus are known for causing treatment resistant epilepsy with gelastic seizures (Kerrigan et al. 2017). They can also be the cause of uncontrollable rage that improves after complete surgical resection of the hamartoma (Savard et al. 2003). The neurons of hypothalamic hamartomas express glutamic acid decarboxylase and putatively have ɣ-aminobutyric acid (GABA) as their primary neurotransmitter (Kerrigan et al. 2017).

The anterior cingulate cortex has been considered the location of the core of the human will, the faculty of decisional intention. This is because the ACC is involved where there is conflict between the emotional response of the amygdala and the analytic response of the prefrontal cortex (DeMartino et al. 2006; Felthous 2008a). Disturbances in aggression and impulsivity have been associated with abnormalities in the fronto-limbic network, including the ACC (Mancke et al. 2015; Sebastian et al. 2014).

9.3 Molecular Components of the Glutamate Neurotransmitter System as Potential Drug Targets for Impulsive Aggression

9.3.1 Genetic Studies Implicating Glutamate in Aggression

Glutamate receptor genes have been linked to aggressive behavior. Brodkin and colleagues identified the glutamate receptor AMPA3 gene (*Gria3*) as a candidate quantitative trait locus in a study of aggression in mice directed toward "dangled" intruder mice (Brodkin et al. 2002; Scott and Fredericson 1951). The subunit of the ionotropic glutamate receptor AMPA3, encoded by the mouse Gria3, contributes to excitatory neurotransmission throughout the brain (Ozawa et al. 1998). However, the importance of this or any other single gene in the glutamate system remains to be supported by studies of "knockout mice" (Anagnostopoulos et al. 2001; Miczek 2001). Given the widespread nature of glutamate in the brain, it is likely that the most significant contributions of glutamate receptor genes occur within the discrete pathway that mediates aggressive behavior. Studies using antisense oligonucleotides for glutamate receptors in areas such as the prefrontal cortex, amygdala, and hypothalamus may be more useful approaches for understanding the importance of glutamate receptor genes in aggression.

9.3.2 Glutamate Metabolism and Receptors

Glutamate levels in blood plasma fluctuate with feeding. The blood brain barrier assists in maintaining separate pools of neurotransmitters including glutamate, which if flowing freely into the CNS could cause permanent neurotoxic damage. This separation of glutamate and other neurotransmitters also minimizes "cross-talk" between the central and peripheral nervous systems (Abbott et al. 2010).

In understanding glutamate's role in impulsive aggression and potential therapeutic modifiers, its metabolism and receptors must be addressed. Glutamate is the most abundant and primary excitatory neurotransmitter in the brain. It is dubbed the brain's "master switch" because of its capacity to excite nearly all neurons in the brain (Stahl 2013). In addition to the synaptic roles of glutamate and GABA described within neurocircuits of aggression, neurotransmitters involved in these circuits also reach relatively distant extra-synaptic sites with high-affinity receptors (e.g., glutamate, (Kew et al. 1998), GABA (Oláh et al. 2009), dopamine (Caillé et al. 1996), and serotonin (Hervé et al. 1987). Thus, glutamate and other neurotransmitters can potentially control a large number of target cells monosynaptically, (Haller 2013), an important consideration for psychopharmacology in general (Vizi 1984, 2000), including the anti-impulsive agents to be discussed below.

Glutamate has three main types of receptors all of which are cation channels: N-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA), and kainate receptors respectively, all of which depolarize the surrounding neuronal membrane. Glutamate also activates G-protein coupled receptors, a distinct class designated as metabotropic glutamate receptors (In GluRs) (Takahashi and Miczek 2014). The roles of glutamate in aggression differ depending on the type, subtype, and location of its receptor as well as specific intrinsic and extrinsic conditions.

The most studied for the relationship between glutamate and aggression is the NMDA receptor, which has biphasic effects of its antagonists. At low doses antagonists of NMDA receptors increase aggression whereas higher doses decrease aggression (Takahashi and Miczek 2014). Illustrating potential environmental influences on NMDA subunits, social isolation of mice with resultant increased aggressive behavior is attended by an increase in NR2A and NR2B subunits in the hippocampus yet decreased NR2A expression in the prefrontal cortex (Zhau et al. 2009).

In order for glutamate to function as a neurotransmitter at the NMDA receptor, glycine must also be present (Kemp and Leeson 1993). As glycine is essentially always present at micromolar levels (Kemp and Leeson 1993), which are sufficient to maintain saturation of NMDA receptors (Kew et al. 1998), it is the glutamate released from presynaptic terminals that functions as a neurotransmitter (Kew et al. 1998). In the brain astrocytes remove glutamate from the cleft. Kir4.kt channels maintain a hyperpolarized membrane which drives the uptake of glutamate which otherwise accumulates in the cleft and impairs proper physiological functioning.

9.3.3 Glutamate in the Neurocircuit of Impulsive Aggression

Much of our knowledge of the neurochemistry of aggression is provided by research on animal models. Results can differ based upon animal species and age, type of aggression assessed, and methodology. As previously discussed the neurocircuits of aggressive behavior involve several different areas and tracts of the brain, and multiple neurotransmitters including, in addition to glutamate and GABA, the inhibitors serotonin and oxytocin and the facilitating modulators, catecholamines, vasopressin, neuropeptide y, substance P, cytokines, and testosterone (Fanning et al. 2021). In animal models hyperarousal (affective, reactive, defensive) appears to correspond with impulsive aggression in humans, whereas its opposite hypoarousal (predatory) animal aggression may at least heuristically serve as an analogue of premeditated (proactive) aggression in humans. An area of the brain that has been much investigated using animal models for the study of the neurocircuitry and neurochemistry of aggression is the hypothalamus.

Within the hypothalamus the neurotransmitters acetylcholine, dopamine, GABA, glutamate, noradrenaline, serotonin, substance P, and vasopressin are involved in aggressive behavior and locally controlled by glucocorticoids and testosterone (Haller 2013). In the cat, affective aggression (defensive rage) is thought to be initiated by projections of glutamergic neurons of the mediobasal hypothalamus to NMDA receptors to the periaqueductal gray (Siegel et al. 1999, 2007). The mediobasal hypothalamus is stimulated by substance Pergic neurons from the medial amygdala as well as dopaminergic and noradrenergic neurons (Haller 2013). Glutamate neurons from the basal amygdala also facilitate defensive rage behaviors by projecting to the PAG and acting on NMDA receptors (Siegel et al. 2007). Similarly in the rat, glutamine is involved in attack behavior in the mediobasal hypothalamus (Adams et al. 1993; Haller et al. 1998; Roeling et al. 1993).

Attack behaviors are mediated by substance P thought projecting from the medial amygdala (Halasz et al. 2008, 2009) and arginine vasopressin afferent neurons (Caffrey et al. 2010; Ferris et al. 2008). The rat mediobasal hypothalamus contains both glutamergic and GABAergic neurons, each with this own localization within the mediobasal hypothalamus (Hrabovszky et al. 2005).

The lateral hypothalamus, active in hypoexcitatory predatory aggression in the cat, is like the hyperexcitatory basomedial hypothalamus, stimulated by dopaminergic and cholinergic neurons (Haller 2013). GABAergic neurons are generally inhibitory but can facilitate affective aggression by inhibiting predatory aggression and vice versa. GABAergic neurons from the mediobasal hypothalamus inhibit activity of the lateral hypothalamus. GABAergic neurons from the medial hypothalamus also inhibit the predatory impulse stimulated by the Substance Pergic neurons from the medial amygdala. Both basomedial and lateral areas of the hypothalamus are activated by dopaminergic and cholinergic neurons and both sites are inhibited by serotonin (Haller 2013; Hassanain et al. 2003).

Within the hypothalamus and the PAG, three neurotransmitters function to suppress defensive rage behavior. Serotonin from the brainstem raphe neurons acts

upon 5-HT_{1A} receptors in either the medial hypothalamus or the PAG, especially the dorsolateral aspect (Hassanain et al. 2003; Shaikh et al. 1997; Siegel et al. 2007). μ-opioid receptors are potent suppressors of rage in the PAG, receptors activated upon by enkephalinergic neurons projecting from the central nucleus of the amygdala. As explained above, $GABA_A$ receptors in the medial and lateral hypothalamus reciprocally suppress either defensive or predatory aggression. GABA neurons of unknown origin also suppress defensive rage by acting on $GABA_A$ receptors in the PAG (Siegel et al. 2007; Shaikh and Siegel 1990).

9.3.4 P3 ERP, Glutamate and Impulsive Aggression

In several abnormal mental or behavioral conditions, including impulsive aggression, the amplitude of the P3 ERP is abnormally low. Barratt and colleagues demonstrated that phenytoin, in contrast to placebo, reduces impulsive aggression and concomitantly increases, i.e., normalizes the P3 of impulsively aggressive subjects (Barratt et al. 1997a, b). This raises the possibility that the anti-impulsive aggression effect of phenytoin may be due, at least in part, to its enhancing efficient information processing as reflected by normalization of the P3 ERP.

Glutamate functioning appears to contribute to the P3, particularly the earlier, more frontal component, viz. P3a. The later, more parietal component, P3b, may not be a function of glutamate activity. Hall and colleagues consider the ratio of glutamine (Gln) to glutamate (Ghu) to be the most specific measure of glutamate functioning at the synapse because it corresponds to the relative amount of each. Using proton magnetic resonance spectroscopy ('H MRS) to measure glutamine (Gln), glutamate (Glu) and Gln/Glu, a measure of glutamergic processing, and obtaining frontal P3a (F_3) and parietal P3b (P_z) using an auditory oddball task on 32 healthy subjects, Hall and colleagues found that the frontal P3a amplitude was significantly positively correlated with Gln ($p = 0.02$) and the Gln/Glu ratio ($p = 0.001$), but these measures were not significantly correlated over the parietal-occipital cortices (Hall et al. 2015). The authors suggest that frontal P3a ERP could be useful in approaching the pharmacological treatment of schizophrenia. We suggest this may also apply to the development and use of AIAAs in the treatment of impulsive aggression, also attended by low amplitude P3 which may be restored with phenytoin which decreases glutamergic synaptic excitation (Cunningham et al. 2000). That phenytoin can decrease impulsive aggression, increase the amplitude of P3, and decrease glutamergic transmission, even though increased glutamergicity contributes to the amplitude of P3a, is an apparent paradox to be addressed with future research.

9.3.5 *Glutamate and GABA Levels in the Brain and CSF of Humans with Impulsive Aggression*

Glutamate (glutamate to total creatinine ratios (Glu/tCr)) and GABA levels have been measured using MR spectroscopy in the ACC of female patients with BPD and ADHD, respectively, two conditions characterized by impulsivity, and aggression to test for association of altered Glu/tCr and GABA levels with impulsivity and aggression, respectively (Ende et al. 2016). Study groups were female patients with BPD, ADHS, and a healthy group of control subjects. The ADHD patients manifested significantly lower GABA levels, whereas the Glu/tCR ratio was not significantly different in any of the three groups. Apart from the two disorders that separated the groups, impulsivity as measured by the Barratt Impulsivity Scale (BIS-11, Barratt; Preuss et al. 2008) was associated by differences in the Glu/tCR: GABA balance. Total score of the BIS-11 and Glu/tCR showed a significant positive partial correlation and the total score on the BIS-11 and GABA exhibited a significant negative partial correlation. Aggression as measured by the score on the Brown, Goodwin Lifetime History of Aggression (BGLHA, Brown et al. 1979) did not show a positive correlation with Glu/tCr, but did evince a significant negative partial correlation where controlled for diagnosis (Ende et al. 2016). Note that primary impulsive aggression or IED was not examined in this study.

CSF Glutamate levels are thought to reflect brain glutamate (Coccaro et al. 2013), because the blood-brain barrier actively moves glutamate into the blood from the interstitial brain fluid (Helms et al. 2012) leaving CSF levels at 20% of that in the peripheral circulation (Abbott et al. 2010). Coccaro and colleagues measured glutamate levels in the CSF of 38 healthy subjects (Coccaro et al. 2013). The investigators used multiple measures of aggression and impulsivity: For aggression, the Life History of Aggression assessment (LHA; Coccaro et al. 1997) and the Aggression Factor score from the Buss-Durkee Hostility Inventory (BDHI, Buss and Durkee 1957); for impulsivity, the Barratt Impulsivity Scale-Version II (BIS-II, Patton et al. 1995) and the Eysenck Personality Questionnaire (EPQ, Eysenk and Eysenck 1977). As previously described, Coccaro and colleagues used a data-reduction step to derive composite variables for "aggression," "impulsivity," and "impulsive aggression" (Coccaro and Lee 2010).

They found that CSF Glutamate levels were statistically, positively correlated with composite measures of aggression ($p = 0.004$), impulsivity ($p = 0.062$), and composite impulsive aggression ($p = 0.026$) in all 38 subjects. Positive correlations were found in both personality disordered subjects (28) and healthy volunteers (10), statistically significant only in the PD group, but due to small sample size, not in the HV group for which the magnitude of the correlation was similar. Personality disordered subjects showed correlations for aggression ($n = 28$, $p = 0.004$), composite impulsivity ($p = 0.033$), and composite impulsive aggression ($p = 0.012$).

The authors interpreted these correlations of CSF Glutamate with aggression, impulsivity, and impulsive aggression to support the theory that CNS glutamate facilitates aggressive behavior (Coccaro et al. 2013; Comai et al. 2012) and

suggested a mechanism whereby glutamate stimulates NMDA, AMPA, and metabotropic receptors in the amygdala, the medial hypothalamus, and periaqueductal gray, as is suggested in animal model experiments eliciting defensive rage behavior (Coccaro et al. 2013). Regardless of the specific mechanism, this is consistent with the top-down bottom-up theory of human aggression involving glutamate (Siever 2008).

In contrast to this positive correlation of CSF glutamate with aggression, Lee, Petty, and Coccaro found no association between CSF GABA levels and aggressive behavior. Instead they found CSF GABA levels to show a positive correlation with impulsivity and with a history of suicidal behavior (Lee et al. 2009).

9.4 Anti-Impulsive Aggression Agents and the Glutamate/GABA Balance

No pharmaceutical agent has been FDA approved for the treatment of impulsive aggression or intermittent explosive disorder, yet the disorder is in need of treatment as it can result in disrupted relationships, domestic abuse, assault and even homicide, with potentially dire consequences for those afflicted as well as the targets of their aggression. Five medications have been identified by more than one high quality study as efficacious for impulsive aggression: fluoxetine, phenytoin, valproic acid/duloxetine, carbamazepine/oxcarbazepine, and lithium (Felthous et al. 2013).

These five AIAAs are classified based on heterogeneous applications: SSRI antidepressant (fluoxetine), anticonvulsant (phenytoin), anticonvulsant and mood stabilizer (valproate/divalproex, carbamazepine/oxcarbazepine), and mood stabilizer without anticonvulsant effect (lithium). The pharmacotherapeutic mechanism for their primary use is not well understood for any of these, and not known for their anti-impulsive aggression effects. Moreover, each agent carries a constellation of molecular effects within the CNS, and the pharmacodynamic profile of each is unique. Most research that aims to explain the method of action does so for the drug's primary indication, viz., depressive, bipolar or seizure disorder, not for impulsive aggression.

A hypothesis for clinical aggression that is gaining currency is the "top-down/bottom-up" hypothesis (Siever 2008), which also attempts to explain the efficacy of agents in controlling impulsive aggression (Stahl and Morrisette 2014). Top down corresponds to the controlling neurotransmitters such as serotonin in the frontal lobes and GABA, whereas bottom up refers to excitatory neurotransmitters, glutamate in particular. According to this hypothesis an imbalance between control and excitation can lead to poorly controlled aggression. This can be the result of too little control from the prefrontal cortex or too much excitation from the amygdala. Impulsive aggression may also represent an imbalance of the glutamergic and GABAergic activity in the aggression mediating amygdala-hypothalamus-periaqueductal gray circuits. Of the various effects and proposed mechanisms of

action for each AIAA, this discussion will focus on those that enhance GABAergicity or diminish glutamaticity in the brain.

The balance in the glutamate GABA-glutamine metabolic cycle has been implicated in several mental disorders and behaviors including aggressive behavior. Thus, in examining the possible role of glutamate signaling in aggression, we must also examine the role of GABA, the principal inhibitory neurotransmitter in the mammalian brain (Zhao and Gammie 2014). GABA and glutamate signaling are important in modulating various behaviors. Among other responses, GABA signaling is associated with aggression (Zhao and Gammie 2014). Activation of the $GABA_A$ receptor in the lateral septum (LS) increases aggression in Syrian Hamsters (McDonald et al. 2012). This may seem counterintuitive if GABA is expected to diminish aggression. However, if enhanced GABA signaling also reduces anxiety and fear, and in the case of Syrian hamsters the expression of conditioned defeat; this mechanism may secondarily embolden the animal allowing expression of aggression unfettered by inhibitory anxiety.

9.4.1 Fluoxetine

Serotonin's effect in improving prefrontal cortical activity (New et al. 2004), normalization of orbitofrontal and anterior cingulate functioning with fluoxetine (New et al. 2004), resulting in prefrontally mediated self-control with diminished impulsive aggression (Coccaro et al. 2015b), have been addressed previously and need not be further discussed here. In addition to the control from enhanced prefrontal serotonicity, Coccaro et al. conclude that fluoxetine alters the corticolimbic circuitry by improving amygdala functioning (Coccaro et al. 2011, 2015b). This is suggested by fluoxetine's reduction of Blood Oxygenation Level Dependent (BOLD) responses in the amygdala as measured by functional magnetic resonance imaging (fMRI).

Among the 14 distinct serotonin receptors (Samuels et al. 2015), serotonin receptor subtypes that have been studied with regard to aggression are $5\text{-}HT_{1a}$, $5HT_{1b}$, $5\text{-}HT_{2a}$, and $5\text{-}HT_3$. Of these, $5\text{-}HT_{1a}$ is strongly implicated in anxiety and depression, and increasingly in impulsivity and aggression. By promoting membrane hyperpolarization, the $5\text{-}HT_{1a}$ receptor mediates inhibitory action on two relevant neuronal populations: raphe serotonergic neurons where autoreceptors are distributed in a somato-dendritic pattern, and serotonergic cortical, hippocampal, and septal neurons postsynaptically (Rosell and Siever 2015). Impulsive aggression is thought to be inhibited by $5\text{-}HT_{1a}$ but promoted by $5\text{-}HT_{2a/6}$ neuromodulation (Lee and Coccaro 2019). The antidepressant action of SSRIs is thought to be due to increased signaling of the $5\text{-}HT_{1a}$ in the dentate gyrus of the hippocampus (Samuels et al. 2015) and chronic SSRI treatment, possibly through post receptor desensitization, reduces cortical $5\text{-}HT_{2a}$ binding (Lee and Coccaro 2019), a two-pronged mechanism that could as well conceivably contribute to SSRIs' action as an AIAA. On the other hand, another SSRI citalopram has been shown by single-

photon emission computed photography (SPECT) to improve the behavior of eight out of nine impulsive aggressive dogs and to decrease binding at the 5-HT_{2a} receptors in cortical, but not subcortical regions (Peremans et al. 2005).

The dorsal raphe nucleus, implicated in modulating aggression and emotions, contains most of the brain's 5-HT neurons (Takahashi and Miczek 2013) which project to the cortex and limbic targets (Takahashi and Miczek 2013). Within the dorsal raphe nucleus, serotonin, the main neurotransmitter of the DRN (Michelsen et al. 2007), and GABA interact in ways that can promote or inhibit aggression depending on their receptor subtype, location and specific conditions (e.g., after alcohol consumption). For example, 5-HT is increased in the medial prefrontal cortex by $GABA_B$ activation in the DRN, suggesting that intermale aggression is attended by activation of DRN 5-HT neurons (Takahashi et al. 2010).

Fluoxetine may also reduce impulsive aggression by increasing allopregnanolone (Allo) which is a positive allosteric modulator of GABAergicity at $GABA_A$ receptors. Research shows that the intensity of aggression shown by socially isolated mice to an intruder is related to the downregulation of Allo content that accompanies social isolation. Fluoxetine both normalizes Allo levels and abolishes aggression against an intruder (Pinna et al. 2003).

Frizzo proposes that fluoxetine and sertraline, another SSRI shown to reduce impulsive aggression Buttler et al. 2010; Feder 1999), may serve as glutamergic modulators by reducing glutamate uptake from the synaptic cleft (Frizzo 2017). Fluoxetine and sertraline inhibit Kir4.lkt channels. The peripheral measure of glutamate uptake in human platelets is thought to reflect similar SSRI reduction in CNS astrocyte uptake of glutamate. If accumulated intersynaptic glutamate is involved in SSRIs mechanism of action, as Frizzo suggests, this is counterintuitive and not fully explanatory, as one would expect proportionate increase, not decrease, in glutamergicity to be associated with impulsive aggression. See Table 9.1 for a summary of the identified effects through animal models of each AIAA on brain glutamate.

9.4.2 Valproate/Divalproex

Structurally distinct from other psychotropic agents, valproic acid (dipropylacetic acid) was used as a solvent in identifying other potential anti-epileptic agents (Bowden 2004) before its anti-epileptic, mood-stabilizing, and anti-impulsive aggression qualities became known. Potential mechanisms of action include neuronal stabilization by reducing sodium influx and increasing potassium efflux, increasing dopamine turnover (Löscher 1993), decreasing aspartate release, decreasing somatostatin in the CSF, and decreasing NMDA-mediated circuits (Bowden 2004).

In line with the up-down/bottom-up hypothesis of aggression, and the GABA/glutamate balance in particular, is the evidence that valproate enhances GABA functioning by increasing its CNS levels (Patsalos and Lascelles 1981), inhibiting the catabolism of GABA, promoting its release, decreasing its turnover, increasing

Table 9.1 AIAAs effects on Glutamergic and GABAergic activity

AIAA	Glutamergic Effect(s)	GABAergic Effect(s)
Fluoxetine	Reduces glutamate uptake from the synaptic cleft by inhibiting Kir4.lkt channels involved in astrocytic uptake of fluoxetine	
Valproate/ Divalproex	Valproate stimulates glutamine synthetase (GS), synthesis of glutamine from glutamate in astrocytes, which is then transported to neurons within which it is transformed into GABA and glutamate	Valproate enhances GABA functioning by inhibiting the catabolism of GABA, promoting its release, decreasing its turnover, increasing $GABA_B$ receptor density and possibly increasing neuronal responsiveness to GABA
Carbamazepine/ Oxcarbazepine	Blocks sodium channels by decreasing glutamate and aspartate release.	Carbamazepine enhances GABA functioning by increasing $GABA_B$ receptors and decreasing GABA turnover.
Phenytoin	Decreases glutamergic synaptic excitation, reduces the frequency of excitatory postsynaptic potentials enhanced by exogenous activation of AMPA and NMDA glutamate receptors. May reduce background and evoked excitation by decreasing the frequency of spontaneous glutamate-mediated EPCSs.	Increases GABAergic synaptic inhibition, increases the level of GABA from presynaptic terminals, increases the amplitude of inhibitory postsynaptic potentials (IPSP) which may indicate postsynaptic potentiations of GABA receptors.
Lithium	Suppresses glutamine-induced loss of phosphorylated CREB caused by the activation of protein phosphatase 1 and antagonizes glutamate-induced activation of c-Jun-N-terminal kinase (JNK), p38 kinase, and AP-1 binding. Effects gene expression and signaling pertaining to excitatory & inhibitory system. Enhances recovery of glutamate-induced Akt inactivation, preventing or reversing extracellular glutamate excitotoxicity.	Increases limbic $GABA_B$ receptors, decreases GABA turnover

$GABA_B$ receptor density and possibly increasing neuronal responsiveness to GABA (Bowden 2004; Post et al. 1992). (See Fig. 9.2.) Valproate may also affect dopaminergic and serotonergic functioning (Löscher 1993) and decrease excessive neurotransmission by reducing the flow of ions through voltage-sensitive sodium channels (VSSC, Stahl 2013).

Whether valproate affects brain and CSF concentrates of glutamate and glutamine depends on the species (Cotariv et al. 1990; Godin et al. 1969; Löscher 1993). Valproate increased concentrations of glutamine, the amino acid precursor for glutamate, in the baboon CSF (Valin et al. 1991), the mouse brain (Kapetanovic et al. 1988), and rat cortex (Patsalos and Lascelles 1981). In rodents treated with

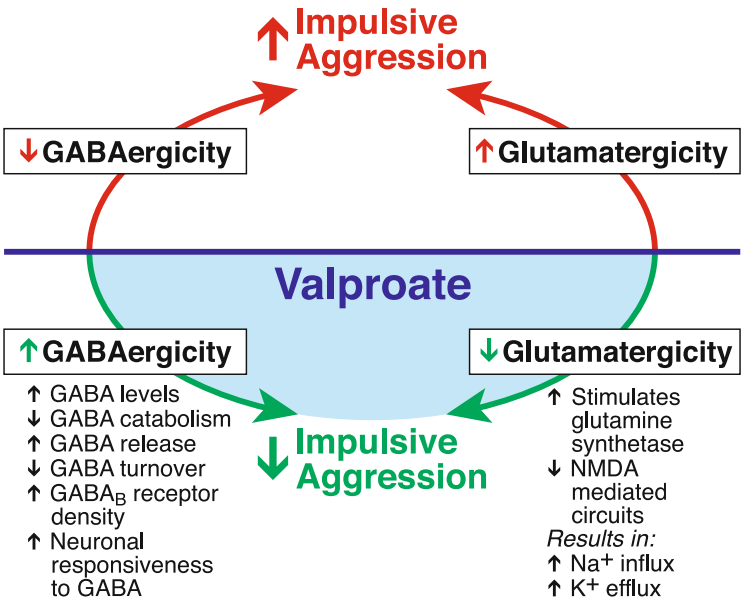

Fig. 9.2 The potential mechanisms whereby valproate reduces impulive aggression illustrates the up down/bottom up hypothesis of aggression. Here the imbalance is illustrated by too little GABA activity and/or too much glutamate activity. Valproate restores the GABA/glutamate balance thereby reducing impulsive aggression

valproate, glutamate concentrations have been reported as increased, decreased, and unchanged (Cotariv et al. 1990; Löscher 1993; Löscher and Hörstermann 1994; Kapetanovic et al. 1988; Patsalos and Lascelles 1981). Kapetanovic and colleagues found that valproate increased basal but decreased newly synthesized GABA. The authors suggested that turnovers of newly synthesized neurotransmitters better reflect their dynamics and neuronal activity. Lowering new GABA by valproate could be the result of feedback inhibition of and decreased synthesis of GABA from increased GABAergic transmission (Kapetanovic et al. 1988). In humans age makes a difference, as valproate increases CSF glutamine in children (Jaeken et al. 1987) but not in adults (Pitkänen et al. 1989).

The effect of valproate on levels of glutamine, glutamate, and GABA is also dependent on the presence of certain mental or neurological disorders. Valproate does not change the level of CSF GABA in patients with epilepsy (Pitkänen et al. 1989), Parkinson's disease (Nutt et al. 1979), or schizophrenia (Lautin et al. 1980). Yet other studies showed an increase in CSF GABA in both epileptic children and adults treated with valproate (Löscher and Siemes 1985; Araki et al. 1988). Petroff et al. (1999) in contrast using nuclear magnetic resonance (NMR) spectroscopy did not find a significant increase in GABA concentration in the occipital lobe of adult patients with complex partial seizures.

9.4.3 Carbamazepine/Oxcarbazepine

The drug of choice for complex partial seizures/temporal lobe epilepsy and tonic-clonic seizures (Mattes 1986; McNamara 2011) is carbamazepine, beginning in the 1980s as one of the first alternatives to lithium in the treatment of bipolar disorder. Now recognized as an effective AIAA, its preclinical anticonvulsant profiles are similar to that of phenytoin and somewhat like that of valproic acid. Like phenytoin, carbamazepine, as well as its metabolite, 10,11-epoxycarbamazepine, limits sustained neuronal firing at therapeutic concentrations. Reduction in neuronal firing appears to be mediated by slowing the rate of recovery from inactivation of voltage-activated sodium channels. At therapeutic levels this is not affected by iontophoretically applied GABA or glutamate (McLean and Macdonald 1986; McNamara 2011).

Of its numerous cellular and intracellular effects, we here concentrate on those that most compare with the effects of lithium and valproic acid, which are also effective AIAAs, and that may affect the GABAergic-glutamergic balance of the up-down bottom-up hypothesis of aggression. Like lithium and valproic acid, carbamazepine increases limbic ɣ-aminobutyric acid (GABA) type B receptors. All three AIAAs increase hippocampal $GABA_B$ receptors in rats (Motohashi 1992; Motohashi et al. 1989) and decrease GABA turnover (Bernasconi 1982; Bernasconi and Martin 1979; Bernasconi et al. 1984). Translationally, Ketter and colleagues suggest that these agents stabilize mood by decreasing GABA turnover and enhancing hippocampal $GABA_B$ receptor mechanisms.

Other effects are in common with either lithium or carbamazepine but not both. Of relevance to the GABA-GLU hypothesis, like valproic acid but unlike lithium, carbamazepine blocks sodium channels thereby decreasing glutamate and aspartate release. Aspartate is the other of the two major excitatory amino acids in high concentration in the CNS (Nester et al. 2001).

9.4.4 Phenytoin

Phenytoin is neither an SSRI nor a mood stabilizer. As an anticonvulsant, phenytoin (diphenylhydantoin, DPH) is effective in the treatment of partial and tonic-clonic but not absence seizures (McNamara 2011). Research on mechanisms of action has the translational goal of explaining phenytoin's anticonvulsant action. Because both seizure disorders and impulsive aggression are disorders of dysregulated neuroexcitement, it is conceivable that explanations of phenytoin's anticonvulsant action may pertain to its therapeutic effect on impulsive aggression as well. If phenytoin improves the GABAergic-glutamergic balance, this may reduce the likelihood of impulsive aggressive outbursts as well as epileptic seizures.

The anticonvulsant effect of DPH is thought to be due to its blockade of Na+ channels. By slowing the rate of recovery of voltage-activated sodium channels from

inactivation, DPH like carbamazepine sustains depolarization and limits neuronal firing (McNamara 2011; McLean and Macdonald 1986). However, research with animal models has demonstrated that DPH both increases GABAergic synaptic inhibition and decreases glutamergic synaptic excitation (Cunningham et al. 2000). Prolonged treatment of rats with DPH resulted in reduced GABA concentration in the cerebellum, hypothalamus, and striatum (Patsolos and Lascelles 1981). DPH appears to both increase the release of GABA from presynaptic terminals and increase the amplitude of inhibitory postsynaptic potentials (IPSPs), which may indicate postsynaptic potentiation of GABA receptors (Cunningham et al. 2000).

While enhancing the GABAergic inhibitory actions, DPH has been shown to reduce the frequency dependent excitatory postsynaptic potentials enhanced by exogenous activation of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NMDA (N-methyl-D-aspartate) glutamate receptors. Although DPH does not affect the regional concentration of glutamate in rats (Patsalos and Lascelles 1981), Cunningham and colleagues demonstrated that phenytoin decreased the frequency but not the amplitude of spontaneous glutamate-mediated EPCSs in the entorhinal cortex neurons of male Wistar rats (Cunningham et al. 2000). They concluded that phenytoin's anticonvulsive efficacy is due to its enhancement of GABA mediated background and evoked inhibition together with its reduction of background and evoked excitation. Given the evidence for GABA/glutamate imbalance as a neuromechanism for aggressive behavior, these findings could as well contribute toward understanding phenytoin's mechanisms for reducing impulsive aggression.

9.4.5 The Non-AIAA Levetiracetam

Levetiracetam ((S)-α-ethyl-2-exo-1-pyrrolidine acetamide) is an effective anti-epileptic drug for pharmacotherapy of myoclonic, partial, and generalized seizure disorder (McNamara 2011). Unlike the AIAAs phenytoin, valproate/divalproex and carbamazepine/oxcarbazepine, which are also anti-epileptics, controlled drug trials have shown levetiracetam to be ineffective in controlling impulsive aggression (Felthous et al. 2013; Mattes 2008). Therefore, potential anticonvulsant mechanisms of action may be usefully compared with those of efficacious AIAAs, in searching for mechanisms specific for controlling impulsive aggression.

By several mechanisms levetiracetam acts on ion channels and inhibits the amplitude of excitatory, postsynaptic currents (EPSC). Levetiracetam interacts with presynaptic P/Q-type voltage-dependent calcium channel (VDCC) to reduce glutamate release. Levetiracetam in contrast to valproate, carbamazepine and phenytoin, has no direct effect on glycine-gated currents (Rigo et al. 2002).

Two important anti-epileptic mechanisms are reducing glutamate release (Cunningham et al. 2004) and blocking glutamate receptors resulting in diminished neuroexcitability (Lee et al. 2009). Using male Wistar rats, Lee and colleagues investigated whether levetiracetam regulates AMPA and NMDA receptor-mediated

excitatory transmission in the dentate gyrus when seizure activity is regulated. Results suggested that levetiracetam modulated the presynaptic P/Q-type voltage-dependent calcium channel. This reduced glutamate release and diminished the amplitude of the EPSC. The authors indicate that this likely contributes to the anti-epileptic therapeutic effect of levetiracetam (Lee et al. 2009). In common with the AIAA anti-epileptic agents, levetiracetam diminishes CNS glutamate activity, but by a different mechanism, presynaptically, and so far demonstrated only in the dentate gyrus of the hippocampus.

9.4.6 Lithium

Lithium, one of the first elements created in the universe, is FDA approved and used primarily in the treatment of bipolar disorder. The lightest alkali metal and a monovalent cation (Baldessarini 1996; Ward et al. 1994), lithium has also been shown effective in the treatment of impulsive aggression (Felthous et al. 2013; Lee et al. 2019; Sheard et al. 1976). Among its multiple effects, lithium influences secondary messenger systems such as cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP). It has been hypothesized that lithium's mood-and-behavior-stabilizing effects are due to lithium's competition with cations of neurophysiological importance, such as sodium, potassium, and calcium (Ward et al. 1994).

Among its effects on multiple neurotransmitter systems are several mechanisms by which lithium may diminish the excitatory effect of glutamate or enhance the inhibitory effects of GABA and acetylcholine. Importantly lithium suppresses glutamine-induced loss of phosphorylated CREB caused by the activation of protein phosphatase 1. Lithium also antagonizes glutamate-induced activation of c-Jun-N-terminal kinase (JNK_1), p 38 kinase and AP-1 binding (Chuang 2004). Gao and colleagues have demonstrated that long-term treatment of cultured rat cerebellar granule cells with lithium increases the m_3-muscarinic acetylcholine receptor-mediated phosphoinositide turnover as well as the levels of c-fos and m_3-receptor mRNA (Gao et al. 1993). Included in lithium's multiple sites of action, therefore, are also sites of gene expression and signaling (Chuang 2004), which also affect the balance between excitatory and inhibitory systems, potentially favoring the control of impulsive aggression.

Excessive accumulation of extracellular glutamate results in glutamate excitotoxicity and even neuronal death. In preventing, diminishing, or reversing the processes leading to glutamate excitotoxicity, lithium is a neuro-protective agent (Chuang 2004). By activating protein phosphatases, treatment with glutamate causes a rapid but reversible loss of Akt (Ser 473) (Chalecka-Franaszek and Chuang 1999). Chuang suggests that Akt contributes in mediating against glutamate excitotoxicity, as this is supported by the finding that the complete loss of Akt activity is prevented by long-term lithium treatment which enhances the recovery of glutamate-induced Akt inactivation (Chuang 2004).

9.5 Antipsychotic Agents and Emerging Treatments for Impulsive Aggression

9.5.1 Antipsychotic Agents

In our review of AIAAs, haloperidol was the only antipsychotic tested to diminish impulsive aggression (Felthous et al. 2013). Only one such quality study was identified and its effect on impulsive aggression in children was favorable. Antipsychotics are shown to be effective in controlling aggressive behavior and other symptoms of mania, psychotic agitation, and schizophrenia. We do not recommend antipsychotics as first-line agents against primary impulsive aggression, because of their significant potential for serious side effects and insufficient evidence of efficacy for IA. Nonetheless, antipsychotics are widely used as general antiaggressive agents. Accordingly, we comment on how three of the more favored antipsychotics for aggression interact with the GABA/glutamine balance: risperidone, olanzapine, and clozapine. In schizophrenic subjects, antipsychotic agents may improve NMDA receptor functioning favoring cognition and reducing negative symptoms by acting through the glycine modulatory site (Goff and Coyle 2001). Much of schizophrenic aggression is phenomenologically impulsive (Felthous 2008b) and hypothetically responsive to pharmacotherapy that improves cognition and executive function.

9.5.2 Risperidone

After haloperidol and before olanzapine and clozapine came onto the scene, the atypical antipsychotic risperidone was shown to control aggressive behavior in patients with schizophrenia (Aleman and Kahn 2001) hospitalized mental patients (Chengappa et al. 2000) and as a last resort when AIAAs were insufficiently effective (Moeller and Swann 2007). Different classes of antipsychotic agents target and modulate three ionotropic glutamate receptor subtypes: NMDA ((N-methyl-D-aspartate acid), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), propionic acid (AMP), and Kainate (Ozawa et al. 1998). It has been hypothesized that dysfunctional glutamatergic neurotransmission can contribute to the onset and neuropathology of schizophrenia and other psychotic disorders (Goff and Coyle 2001; Tsai and Coyle 2002). The psychotomimetic agents phencyclidine and ketamine have been shown to act on NMDA receptors (Javitt and Zukin 1991; Tsai and Coyle 2002). As risperidone is an effective antipsychotic and antiaggressive agent, even if not specifically tested for primary impulsive aggression, it is conceivable that both effects are induced by the same disturbance in glutamine transmission.

Using brain homogenates of Sprague-Dawley rats, comparing juvenile and adult rats, Choi and colleagues tested for in vitro glutamate receptor affinity and receptor autoradiography at the three glutamate receptors specified above. Risperidone showed very low affinity for all three glutamate receptors in both juvenile and

adult rat brain homogenate. Weekly injection of high doses of risperidone did not alter the levels of NMDA, AMPA, or KA (kainic acid) receptors in the medial prefrontal cortex, the caudate-putamen or the nucleus accumbens, whereas high doses (1.0 and 3.0 mg/kg/day) significantly decreased NMDA binding in the caudate-putamen of juvenile and adult rats, and in the nucleus accumbens of juveniles but not adults. In juveniles but not adults, these two doses of risperidone decreased NMDA receptors in the nucleus accumbens. Risperidone did not change the number of kainite receptors at either dose in either juveniles or adults (Choi et al. 2009).

Thus, risperidone has different effects at different doses on the glutamate receptors in different areas of the brain and at different phases of animal development. The three ionotropic glutamate receptors behave differently in different brain areas and at different phases of brain development. Accordingly the mechanisms of glutamate in modulating aggression may not be the same in those areas of the brain known to be involved in the release and control of aggressive behavior or for that matter across species.

9.5.3 Olanzapine

One of the most widely used antipsychotic agents for the treatment of acute and recurrent aggression in the schizophrenia spectrum disorders in particular is olanzapine, a thienobenzodiazepine derivative (2-methyl-4(4-methyl-1-piperazine)-10H-thienol[2-3-6][1.5]benzodiazepine) which is chemically related to clozapine, a dibenzodiazepine. Like other antipsychotics it blocks D_2 receptors (Kapur and Remington 2001) and D_2 receptor blockage has been thought to be an important component of the antipsychotic therapeutic action. Atypical antipsychotics also act on 5-HT receptors and olanzapine is a much stronger blocker of 5-HT_{2A} than DA receptors (Kapur et al. 1998). The mechanism of "atypicality" has been attributed to the 5-HT-DA antagonist hypothesis (Meltzer et al. 1989) with the greatest atypicality shown by agents like olanzapine with the relatively greater 5-HT receptor blockade (Schulz et al. 2004).

Because both quetiapine and olanzapine reversed isolation-induced prepulse inhibition effect in rats (Bakshi et al. 1998), and NMDA antagonists are associated with prepulse inhibition, olanzapine appears to have an effect on the glutamergic system which is implicated in the pathophysiology of schizophrenia (Schulz et al. 2004). Using magnetic resonance spectroscopy, Goff and Coyle (2001) found that glutamate levels increased when patients were switched from typical antipsychotic medication to olanzapine. Brain levels of glutamate did not increase except in those patients whose symptoms improved following the switch to olanzapine. These findings are consistent with the hypo-glutamergic hypothesis of schizophrenia (Moghaddam and Javitt 2012).

Uniquely olanzapine, and not clozapine, chlorpromazine, haloperidol, bupropion, fluoxetine, and amitriptyline, inhibits the activity of D-aspartate oxidase. D-aspartate

activation of NMDA receptors has been shown to be at low levels in schizophrenia (Sacchi et al. 2017). D-aspartate stimulates the release of glutamate in the prefrontal cortex of mice. These and other experiments support the possibility that by inhibiting activity, olanzapine increases L-glutamate release in murine prefrontal cortex (Sacchi et al. 2017). Thus, olanzapine may diminish symptoms of schizophrenia, including perhaps secondary aggressive behavior, by *increasing* glutamate availability.

9.5.4 Clozapine

A dibenzepine tricyclic antipsychotic primarily used in the treatment of schizophrenia and schizoaffective disorder and especially refractory schizophrenia (Marder and Wirshing 2004), clozapine has been shown to be much more effective than other antipsychotics in bringing aggressive behavior under control. Following research protocol, clozapine was demonstrated to bring aggression under control in non-psychotic, nonschizophrenic subjects diagnosed with psychopathy whose aggression was non-responsive to more commonly used agents (Brown et al. 2016).

Hippius (1999) noted delay in appreciating the efficacy of clozapine because it did not conform to popular theory of mechanism of action. Clozapine is especially atypical as an antipsychotic in that it is not a neuroleptic with extrapyramidal side effects. Clozapine has low dopamine (D_2) receptor occupancy and so its remarkable efficacy cannot be explained with the dopamine theory of psychosis. Similar to other second generation antipsychotics clozapine's affinity ratio of 5-HT_{2A} to D_2 receptors is high (Marder and Wirshing 2004).

A more recent explanation for clozapine's efficacy in the treatment of schizophrenia, and we might suggest in controlling aggressive behavior, is its effect on glutamate and glutamine levels. Antipsychotic medication decreases glutamate or total glutamate plus glutamate in the brains of patients with schizophrenia as demonstrated by proton magnetic resonance spectroscopy studies (Goldstein et al. 2015). In the study by Goldstein and colleagues, those patients with schizophrenia who responded to first-line antipsychotics had high total glutamate plus glutamine levels in the prefrontal cortex in comparison with those with ultra-treatment resistant schizophrenia. On the other hand, patients with treatment resistant schizophrenia had higher total glutamate plus glutamine levels scaled to creatinine in the putamen than both first-line responders and those with ultra-treatment resistant schizophrenia. Because the patients with schizophrenia who did not respond to first-line antipsychotics but did respond to clozapine showed elevated total glutamate-glutamine levels scaled to creatinine in the putamen, this was considered a marker for favorable response to clozapine (Goldstein et al. 2015). This finding raises the question of whether the finding could also be associated with schizophrenic-associated aggression that is also responsive to clozapine (Table 9.2).

Table 9.2 The effects of antipsychotics on Glutamergic and GABAergic activity

Antipsychotic	Glutamergic Effect(s)	GABAergic Effect(s)
Risperidone	Risperidone may have different effects on glutamate receptors in different areas of the brain and at different phases of animal development	
Olanzapine	Glutamate levels increase in patients whose antipsychotic is switched to olanzapine and subsequently the patient's symptoms of schizophrenia improve, consistent with the hypo-glutamergic hypothesis of schizophrenia. Uniquely, may diminish symptoms of schizophrenia by increasing glutamate activity by inhibiting D-aspartate oxidase activity, enhancing D-aspartate activity, which in turn stimulates the release of glutamate.	
Clozapine	Patients with schizophrenia who did not respond to first-line antipsychotics but who responded to clozapine have higher glutamate-glutamine levels scaled to creatinine.	

9.5.5 Glutamate Antagonists

9.5.5.1 Memantine

Although not applied to primary impulsive aggression, memantine, an N-methyl-D-aspartate antagonist, has been shown to reduce aggressive behavior in patients with moderately severe to severe Alzheimer's disease (Wilcock et al. 2008). From a pooled analysis of three large six-month randomized studies of subjects with moderately severe to severe Alzheimer's disease, Wilcock and others found that memantine in comparison with placebo showed improved cognition, global functioning, activities of daily living, and decelerated deterioration. At week 12 (53.3% vs. 43.1%, $p = 0.011$) and week 24/28 (61.0% vs. 45.0%, $p < 0.001$) a significantly higher percentage of the patients receiving memantine had improvement in their aggression and agitation. That antiglutamergic pharmacotherapy would diminish aggression supports the role of glutamergic neurotransmission in some types of aggression, viz., that which is secondary to Alzheimer's disease.

9.6 Conclusions and Future Directions

Animal models and neuroimaging and lesions studies in humans have elucidated the neurocircuitry of impulsive aggression and the role of the glutamate excitatory neurotransmitter within this neurocircuitry. Although interacting with several other neurotransmitter systems, also within this circuitry, the balance between glutamate and the neuroinhibitory neurotransmitters GABA and serotonin appears to be crucial. From the developing glutamate, GABA, serotonin model of impulsive aggression, possibilities are suggested for mechanisms of medication with evidence of efficacy as AIAAs. Future research in the development of AIAAs with increasing

efficacy and specificity will progress on two fronts: (1) the continued use of animal models including gene knockout glutamate and interacting neurotransmitters and the effect of identified as well as potentially new AIAAs, (2) and well-designed studies of candidate AIAAs on impulsive aggression, its neurocircuitry, and neurotransmitters, with increasing attention to the subtypes of glutamine receptors.

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Chapter 10
Glutamatergic Treatments for Parkinson's Disease

Fabrizio Gardoni

Abstract Parkinson's disease is characterized by the degeneration of dopaminergic neurons of the *substantia nigra pars compacta* (SNpc) projecting to the striatum and resulting motor and non-motor symptoms. The current knowledge demonstrates that the activity of glutamatergic signals from the cortex to the striatum is strictly regulated during the progression of the disease and indicates that modulation of synaptic transmission at the glutamatergic synapse represents a major target to rescue the altered neurotransmission. Molecular and functional alterations of glutamate receptors in experimental models of Parkinson's disease as well as in patients have been demonstrated and several studies have been performed by using receptor antagonists/modulators. In particular, compounds targeting N-methyl-d-aspartate-type (NMDA) glutamate receptors and specific subtypes of metabotropic glutamate receptors (mGluR) have been tested both in preclinical and clinical studies. At present, amantadine, a low-affinity non-competitive NMDA receptor antagonist, represents a recommended add-on agent to decrease the dyskinetic motor complications of the dopaminergic therapy.

The chapter will describe advances in basic research, preclinical and clinical studies in the attempt of identifying innovative strategies for the modulation of glutamate receptors in Parkinson's disease. Overall, these results indicate that modulation of the glutamatergic system remains one of the promising pharmacological strategies in the field.

Keywords Glutamate · NMDA receptor · Striatum · Levodopa · Dyskinesia · Dopamine

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_10

10.1 Introduction

Parkinson's disease is the second most frequent neurodegenerative disorder associated with aging and affects approximately 1% of the population over 65 years, the mean age at which the disease is diagnosed. The disease is characterized by the progressive degeneration of dopaminergic neurons of the *substantia nigra pars compacta* (SNpc) projecting to the striatum and the consequent motor and non-motor impairments. However, other cell types and neurotransmitter systems are also involved, probably from early disease onwards and the underlying pathogenesis involves multiple pathways and mechanisms.

Parkinson's disease is also characterized by abnormal presence of toxic forms of α-synuclein that, when clustered into Lewy bodies, represents one of the pathological hallmarks of the disease. α-synuclein is present physiologically as monomer widely distributed in the brain and is involved in the regulation of presynaptic vesicle pool, neurotransmitter release, synaptic function, and plasticity. However, α-synuclein has the intrinsic tendency to aggregate in structures of higher molecular weights leading to the formation of oligomers, protofibrils, and eventually fibrils, the main components of Lewy bodies (Spillantini et al. 1997; Wong and Krainc 2017). Mechanisms mediating α-synuclein toxicity in humans have not yet been fully determined. However, recent studies point to α-synuclein oligomers and to protofibrils as major players of the synaptic dysfunction as observed in Parkinson's disease.

The natural history of Parkinson's disease is complex and involves differential mechanisms during its various pre-symptomatic and symptomatic phases. Although clinical diagnosis mainly relies on the evaluation of bradykinesia and other motor symptoms, Parkinson's disease is also characterized by the onset of many non-motor symptoms that increase the overall disability of the patient. Accordingly, beyond the notion of Parkinson's disease as a motor disorder, it is now clear that numerous non-motor symptoms such as cognitive impairment, autonomic dysfunction, disorders of sleep, depression, and hyposmia contribute to the disease. Importantly, early clinical symptoms of Parkinson's disease are detected only when about 70–75% of dopaminergic neurons are already lost.

10.2 Cross-Talk Between Dopamine and Glutamate Receptors

An integrated cross-talk between dopamine and glutamate receptors plays an essential role in driving a physiological motor behavior. In particular, dopaminergic terminals from SNpc converge with glutamatergic terminals at dendritic spines of striatal GABAergic spiny projection neurons (SPNs), that represent more than 95% of striatal neurons. Accordingly, in the corpus striatum dopamine release from SNpc

dopaminergic neurons strictly regulates the activity of corticostriatal glutamatergic inputs onto the striatal SPNs and therefore controlling the overall striatal output.

In the last decades, several possible mechanisms have been proposed to explain dopamine-mediated regulation of the glutamatergic synapse. Early reports indicated that dopamine modulates the function of ionotropic glutamate receptors leading with a reduction of AMPA-type receptor-evoked currents and an increase of NMDA-type receptor-evoked currents (Cepeda et al. 1993; Levine et al. 1996; Cepeda and Levine 1998).

In the striatum, SPNs express NMDA receptor subtypes containing GluN2A and GluN2B regulatory subunits. However, unlike many other adult brain regions, GluN2B is the main GluN2-type subunit at SPN synapses (Dunah and Standaert 2001). Interestingly, GluN2A and GluN2B subunits differentially contribute to the glutamatergic transmission in striatal SPNs (Paoletti et al. 2008; Jocoy et al. 2011). While blockade of GluN2A increases D1 dopamine receptor-mediated potentiation of NMDA receptor responses, inhibition of GluN2B reduces this potentiation, suggesting a counterbalance of their respective functions. Moreover, it has shown that GluN2A subunits contribute mainly to NMDA responses in SPNs containing the D1 receptor, whereas GluN2B subunits are more involved in NMDA responses in SPNs containing the D2 receptor (Paoletti et al. 2008; Jocoy et al. 2011). D1 receptor activation can also play important roles in the modulation of NMDA receptor subunit localization/trafficking at the postsynaptic membrane (Hallett et al. 2006; Dunah et al. 2004; Tang et al. 2007; Vastagh et al. 2012).

Notably, other studies describing the co-localization of D1 and NMDA receptors at SPN synapses put forward the idea of a direct interaction between the two receptors (Kung et al. 2007; Heng et al. 2009; Kruusmägi et al. 2009; Jocoy et al. 2011; Vastagh et al. 2012). In particular, D1-type dopamine receptors directly interacts with the GluN1 obligatory subunit of NMDA receptors with the formation of functional heteromeric complexes at striatal SPN synapses (Cahill et al. 2014; Fiorentini et al. 2003; Lee et al. 2002; Pei et al. 2004). Interaction with NMDA receptors recruits D1 receptors to the synaptic membrane and limits its agonist-induced internalization (Fiorentini et al. 2003; Pei et al. 2004; Scott et al. 2006). Interestingly, disruption of D1 receptor binding to GluN1 by treatment with cell-permeable peptides abolished the synaptic retention of D1 receptors, thus suggesting that D1 are enriched at glutamatergic synapses through a mechanism requiring the interaction with NMDA receptors (Ladepeche et al. 2013a). Moreover, disruption of D1/NMDA receptor complex modulates NMDA receptor synaptic levels through lateral redistribution and promotes long-term potentiation (Ladepeche et al. 2013b). D2 receptors interact specifically with GluN2B-containing NMDA receptors (Liu et al. 2006) and dopamine stimulation by treatment with cocaine enhances the formation of this complex and inhibits NMDA receptor-mediated currents in SPNs (Liu et al. 2006).

As mentioned above, dopamine can also modulate the activity of AMPA receptors. Early studies showed that activation of D1 receptors can promote the phosphorylation of AMPA receptors by protein kinase A, the increase of AMPA receptor surface expression (Snyder et al. 2000; Gao et al. 2006; Vastagh et al. 2012), and the

potentiation of AMPA receptor currents (Price et al. 1999). Conversely, D2 receptor agonists can decrease AMPA receptor phosphorylation (Håkansson et al. 2006).

10.3 The Glutamatergic System in Disease Setting

The degeneration of the nigrostriatal dopaminergic pathway that occurs in Parkinson's disease leads to significant morphological and functional modifications of the corticostriatal glutamatergic synapse and of the striatal neuronal circuitry. Most of the preclinical and clinical results obtained in the last decades have clearly demonstrated that the subcellular localization and the activity of postsynaptic glutamate receptors at striatal SPNs represent a key event in the pathogenesis of Parkinson's disease as well as in the onset of drug-induced motor complications. Different types of modifications of both ionotropic and metabotropic glutamate receptors in striatum have been described in several different experimental models and in postmortem specimens from Parkinson's disease patients (Gardoni and Di Luca 2015; Mellone and Gardoni 2018). Accordingly, it has been put forward the idea that the rescue of a physiological glutamatergic activity in the striatum could represent a useful strategy to restore functional alterations of the basal ganglia circuitry in the different phases of Parkinson's disease.

10.3.1 Alterations of NMDA Receptors in Parkinson's Disease and L-DOPA-Induced Dyskinesia

NMDA receptor dysfunction has been described in several brain disorders, including ischemia, neuropathic pain, schizophrenia, addiction, and neurodegenerative diseases (Paoletti et al. 2013). In Parkinson's disease and after chronic treatment with L-DOPA, the glutamatergic signaling from the cortical afferents to the striatum undergoes adaptive changes leading to an excessive release of glutamate from the presynaptic terminals together with an aberrant distribution and activity of NMDA receptors at dendritic spines of SPNs (Mellone and Gardoni 2018).

NMDA receptor subunit composition varies across the different brain areas and it influences the receptor biophysical and pharmacological properties (Paoletti et al. 2013; Sanz-Clemente et al. 2013). Moreover, NMDA receptor subunit composition strictly regulates NMDA receptor distribution within synaptic or extra-synaptic sites and this event closely affects NMDA receptor activity and intracellular signaling (Hardingham and Bading 2010). In Parkinson's disease, alterations in the levels of NMDA receptor GluN2-type regulatory subunits at the postsynaptic membrane are associated with the extent of dopamine denervation as well as with the development of dyskinesia after chronic treatment of patients with L-DOPA (Hallett et al. 2005; Mellone et al. 2015; Paillé et al. 2010). Early preclinical studies found a decrease of

the GluN2B subunit in striatal membranes of the rat model of the disease (Dunah et al. 2000; Gardoni et al. 2006). Interestingly, dopamine depletion induces similar alterations in the levels of striatal GluN2B-containing receptors at synapses of parkinsonian macaques (Hallett et al. 2005). Conversely, an increased expression of synaptic GluN2A was observed in a model characterized by a partial (about 75%) lesion of the nigrostriatal pathway and mild motor symptoms (Paillé et al. 2010), suggesting that different degrees of dopamine denervation could lead to specific alterations of the NMDA receptor. Notably, the above-described alterations in NMDA receptor subunit composition at SPNs synapses correlated with the reduction of NMDA receptor-dependent corticostriatal synaptic plasticity (Gardoni et al. 2006; Picconi et al. 2004).

More recent *in vitro* and *in vivo* studies showed α-synuclein-dependent modifications of NMDA receptors. In primary cultured neurons, α-synuclein modulates the levels and the function of GluN2B-containing NMDA receptors (Navarria et al. 2015). In cultured dopaminergic cells, α-synuclein increases clathrin-mediated endocytosis of NMDA receptors through the participation of Rab5B (Cheng et al. 2011). Similar results were obtained in primary neurons treated with recombinant human α-synuclein or overexpressing α-synuclein (Chen et al. 2015). Interestingly, α-synuclein can also interact with the prion protein PrPC and triggers calcium dyshomeostasis and synaptic damage through a mechanism involving activation of Fyn kinase, mGluR5 and GluN2B-containing NMDA receptors (Ferreira et al. 2017). *In vivo* injection of α-synuclein decreases NMDA receptor-mediated synaptic currents and impairs corticostriatal long-term potentiation of striatal SPNs (Diógenes et al. 2012; Durante et al. 2019). Notably, treatment with antibodies targeting α-synuclein prevents the α-synuclein-induced effects on the glutamatergic corticostriatal synapse suggesting that this strategy might counteract synaptic dysfunction occurring in Parkinson's disease (Durante et al. 2019).

Cholinergic interneurons (ChIs) represent a very small portion (about 2%) of neurons in the striatum, but they are major players in striatal neurotransmission, regulating both dopamine and glutamate inputs to the striatum (Lapper and Bolam 1992; Ding et al. 2010). In the adult, ChIs selectively express NMDA receptors containing GluN2B/GluN2D regulatory subunits (Bloomfield et al. 2007; Tozzi et al. 2016). In physiological conditions, striatal GluN2D-containing NMDA receptors contribute to the inhibition of both dopamine and glutamate release through the action of acetylcholine released by ChIs (Zhang et al. 2014). However, this inhibitory function on the glutamatergic transmission is altered in the striatum of a mouse model of Parkinson's disease, suggesting that this NMDA receptor subtype may play a role in the adaptive changes occurring in the disease (Feng et al. 2014). Moreover, in the disease also SPNs start to express GluN2D-containing receptors, thus resulting in complex modification of NMDA receptor-mediated synaptic transmission (Zhang and Chergui 2015). Overexpression of α-synuclein blocks the induction of long-term potentiation in ChIs, producing early memory and motor alterations. Notably, these effects are dependent on α-synuclein modulation of the GluN2D-expressing NMDA receptors (Tozzi et al. 2016). However, despite the emerging function of GluN2D

subunit in the disease process, further investigation is still needed before compounds specifically targeting this NMDA receptor subtype can find a clinical application.

Changes of the synaptic levels of NMDA receptor subunits are strictly correlated with modifications of their binding with scaffolding proteins, namely members of the membrane-associated guanylate kinase (MAGUK) protein family, i.e. PSD-95 (Gardoni et al. 2006). Interestingly, a decreased synaptic membrane localization MAGUK proteins and interaction with GluN2-type subunits has been reported in experimental parkinsonism thus addressing a key role also for MAGUK/NMDA receptor clustering in the observed aberrant localization of NMDA receptor subunits at synapses (Gardoni et al. 2006, 2012; Picconi et al. 2004). Finally, genome-wide studies identified *GRIN2A* gene, encoding the GluN2A subunit, as a genetic modifier of the inverse association of coffee with the risk of developing Parkinson's disease (Hamza et al. 2011; Simon et al. 2017). In addition, another study indicated that vertebrate motor behavior and synaptic signaling acquired depend upon the duplication and diversification of ancestral GluN2-type genes (Ryan et al. 2013).

Alterations of the synaptic enrichment of both GluN2A and GluN2B subunits of the NMDA receptor have been widely described in both rat and monkey models of dyskinesia following chronic L-DOPA administration (Gardoni et al. 2006, 2012; Mellone et al. 2015). In particular, a prolonged exposure to L-DOPA induces a redistribution of GluN2B-containing receptor to the extra-synaptic membrane, and a concomitant increase of GluN2A synaptic levels in striatal SPNs. This leads to a significant increase of NMDA receptor GluN2A/GluN2B ratio at SPN dendritic spines. Notably, these results have been further confirmed in the putamen of postmortem tissue from Parkinson's disease patients showing a high dyskinetic profile (Mellone et al. 2015). Overall, these reports suggest that an imbalance in the synaptic pool of specific NMDA receptor subtypes in the striatum is responsible for the disturbances of the glutamatergic synapse underlying L-DOPA-induced dyskinesia. Importantly, all these changes in synaptic NMDA receptor subunit content in striatal SPNs correlate with the motor behavior abnormalities and the altered expression of striatal plasticity observed in experimental models of both Parkinson's disease and L-DOPA-induced dyskinesia (Gardoni et al. 2006; Paillé et al. 2010; Picconi et al. 2004). Even if the exact mechanisms leading to this aberrant NMDA receptor synaptic retention are not fully elucidated, several reports identified alterations of the interaction of GluN2 subunits with specific binding partners, i.e. different types of scaffolding proteins. Among others, MAGUK proteins such as PSD-95, SAP97 and SAP102 show a reduced association with GluN2B in dyskinetic rats (Gardoni et al. 2006), while an increased interaction with PSD-95 and Rph3A may be responsible for the augmented GluN2A anchoring at the postsynaptic density of striatal SPNs (Mellone et al. 2015; Stanic et al. 2017). Other studies suggest that dyskinesia onset is related to the increase in PSD-95 and SAP97 at the synaptic membrane of experimental rodent and primate models (Nash et al. 2005; Porras et al. 2012).

Besides NMDA receptor activity and subunit composition, other modifications such as phosphorylation of specific subunit have been found altered in experimental models of dyskinesia. In particular, GluN2B subunit Tyr1472 phosphorylation is

increased in different animal models of dyskinesia and this is believed to affect AP-2-mediated endocytosis of GluN2B-containing NMDA receptors (Oh et al. 1998; Quintana et al. 2010).

10.3.2 Alterations of AMPA Receptors in Parkinson's Disease and L-DOPA-Induced Dyskinesia

AMPA receptors are ionotropic glutamate receptors involved in several brain functions including synaptic transmission, spine morphology, and synaptic plasticity (Huganir and Nicoll 2013). Alterations in the molecular mechanisms that regulate AMPA receptor assembly and trafficking have been found in neurological and neurodegenerative disorders (Henley and Wilkinson 2016). In particular, impairments in AMPA receptors synaptic localization and phosphorylation have been observed in preclinical models of L-DOPA-induced dyskinesia and in Parkinson's disease patients. Importantly, enhanced phosphorylation of AMPA receptors GluA1 subunit on serine845 by PKA has been described in rodent and non-human primate models and it has been defined as an experimental marker of dyskinesia (Ba et al. 2006; Errico et al. 2011; Santini et al. 2007, 2010). Moreover, modifications of AMPA receptor subunit composition have been also demonstrated (Hallett et al. 2005; Silverdale et al. 2010). Finally, excessive AMPA receptor activity has been correlated to the development of dyskinesia in animal models (Kobylecki et al. 2010; Konitsiotis et al. 2000).

10.3.3 Alterations of Metabotropic Glutamate Receptors in Parkinson's Disease and L-DOPA-Induced Dyskinesia

Activation of metabotropic glutamate receptors (mGluRs) plays a key role for a fine modulation of synaptic transmission and neuronal excitability at the glutamatergic synapse in the brain. A great variety of mGluR subtypes are expressed in the different brain area (mGluR1-8) giving the opportunity for a selective pharmacological intervention in those pathological conditions characterized by specific mGluR subtype alterations (Crupi et al. 2019). mGluR4 and mGluR5 represent the two mGluR subtypes that have been mostly correlated to Parkinson's disease pathogenesis as well as to the onset of L-DOPA-induced dyskinesia in the last fifteen years. In the striatum, mGluR4 are located at presynaptic membranes of glutamatergic synapses and their activation induces a decrease in neurotransmission at the striatopallidal synapse (Bogenpohl et al. 2013; Valenti et al. 2003, 2005). In particular, its activation determines a reduction in GABA and glutamate release in the indirect pathway of the basal ganglia (Conn et al. 2005). Accordingly, several

studies identified mGluR4 as a potential target for the control of motor symptoms in Parkinson's disease. Indeed, the group III mGluR-selective agonists inhibit striatum-evoked GABAA-mediated current recorded in the neurons of the globus pallidus through a presynaptic mechanism mediated by mGluR4 (Valenti et al. 2003). This finding indicates that mGluR4 may selectively modulate striatopallidal transmission and that activation of mGluR4 may decrease the excessive inhibition of the globus pallidus in Parkinson's disease.

mGluR5 is located at the postsynaptic membranes of striatal SPNs, where they allow for a fine regulation of the activity of NMDA receptors (Gubellini et al. 2004; Rouse et al. 2000). Several preclinical reports demonstrated that enhanced mGluR5 levels in the striatum may contribute to the pathogenesis of L-DOPA-induced dyskinesia both in the rodent and in the monkey model (Ouattara et al. 2010; Samadi et al. 2008). Interestingly, pharmacological prevention of L-DOPA-induced-dyskinesia is strictly correlated with a decrease of mGluR5 binding. In addition, analysis of mGluR5 mRNA levels and specific binding after chronic L-DOPA treatment and withdrawal confirm that fluctuating levels of mGluR5 can contribute to the development of dyskinesia (Ouattara et al. 2010).

10.4 Current Treatments and Clinical Use of Modulators of Glutamatergic Signaling for Parkinson's Disease and L-DOPA-Induced Dyskinesia

The degeneration of nigrostriatal dopaminergic neurons leading to reduction of striatal dopamine levels is the key mechanism underlying the main motor features of Parkinson's disease (Poewe et al. 2017). Accordingly, the systemic administration of the dopamine precursor levodopa (L-DOPA) represented a huge breakthrough in the treatment of the disease. More recently, several step forwards in the understanding of the pharmacological regulation of dopaminergic neurotransmission have revealed multiple additional targets for dopaminergic therapies. Monoamine oxidase type B (MAO-B) and catechol-O-methyltransferase (COMT) inhibitors and dopamine receptor agonists are now available for clinical use, very often as add-on treatment to L-DOPA, that remain the gold standard therapy for Parkinson disease, and over time, almost all patients with this disease will require L-DOPA administration. However, chronic treatment with L-DOPA is associated to the onset of motor complications, including motor oscillations and L-DOPA-induced dyskinesia. Even if the cellular and molecular mechanisms involved in these side effects are still not fully understood, both presynaptic and postsynaptic mechanisms play a relevant role. In particular, as described above, excessive glutamatergic neurotransmission and aberrant NMDA receptor activity play a key role in the genesis of L-DOPA-induced dyskinesia. At present, amantadine is the only evidence-based medicine review recommended for the treatment of dyskinesia from the Movement Disorders Society (Hubsher et al. 2012; Perez-Lloret and Rascol 2018). Amantadine is a

non-competitive, low-affinity, NMDA receptor antagonist at the phencyclidine binding site which has also been shown to block cholinergic muscarinic receptors, to increase dopamine release and to inhibit its reuptake (Hubsher et al. 2012). However, numerous studies suggest that the anti-dyskinetic effect observed with the clinical use of amantadine is mostly related to its NMDA receptor blocking properties (Perez-Lloret and Rascol 2018). Amantadine is a synthetic tricyclic amine that belongs to the class of aminoadamantanes (Deleu et al. 2002), which was originally used in the 1960s for addressing viruses and treating influenza (Hubsher et al. 2012). From the late 1990s, clinical studies started to support its use in Parkinson's disease patients. An initial evaluation of the efficacy of amantadine on dyskinesia, performed in a small placebo-controlled cross-over study in 18 Parkinson's disease patients with motor fluctuations and dyskinesia (Verhagen Metman et al. 1998), showed that amantadine is able to reduce the severity of dyskinesia without modifying motor symptoms of the disease. The anti-dyskinetic effect was still present one year after as assessed in a placebo-controlled follow-up study in patients still receiving amantadine, thus indicating a prolonged effect of the drug on dyskinesia (Metman et al. 1999). However, another randomized, placebo-controlled study in 40 Parkinson's disease patients with motor fluctuations suggested an effect of amantadine treatment lasting for less than 8 months (Thomas et al. 2004). Notably, withdrawal of amantadine induced a rebound with increase of dyskinesia in 11 patients. These conflicting results prompted to assess the long-term anti-dyskinetic effect of amantadine in other randomized placebo-controlled parallel-group studies (Ory-Magne et al. 2014; Wolf et al. 2010). In the study performed by Wolf and colleagues, patients were treated with amantadine for at least one year and then switched in a double-blind manner to amantadine or placebo. Ory-Magne and colleagues switched patients to either amantadine or placebo after at least 6 months of stable treatment with amantadine. In both trials, dyskinesia worsened significantly in patients receiving placebo but not in those treated with amantadine. Overall, these studies suggested long-term anti-dyskinetic effects of amantadine in Parkinson's disease patients with dyskinesia induced by L-DOPA and prompted the evaluation and the clinical use of different pharmaceutical forms of amantadine (currently available in the market): an oral immediate-release, an extended-release, and an intravenous infusion (Perez-Lloret and Rascol 2018).

Amantadine is not the only drug used in Parkinson's disease patients targeting the glutamatergic synapse. Safinamide is a drug recently approved first by the European Commission and more recently by the US Food and Drug Administration (FDA) as an adjunctive treatment to L-DOPA in patients with mid- to late-stage Parkinson's disease and motor fluctuations. The pharmacological profile of safinamide includes reversible MAO-B inhibition, blockage of voltage-operated sodium channels, and modulation of voltage-operated calcium channels, which results in an *in vivo* inhibition of glutamate release (Müller and Foley 2017). Accordingly, safinamide is a drug characterized by a broad spectrum of pharmacological actions targeting both dopaminergic and glutamatergic neurotransmission. Results from several clinical trials suggest that safinamide represents a good option for add-on therapy to L-DOPA in patients with advanced Parkinson's disease with motor complications

(Borgohain et al. 2014; Schapira et al. 2017). In particular, these studies have shown that safinamide increased ON time with no or non-troublesome dyskinesia, decreased daily OFF time, improved overall motor function and quality of life (Borgohain et al. 2014; Schapira et al. 2017). However, there is still insufficient evidence to recommend safinamide as monotherapy or add-on therapy in patients with early Parkinson's disease. Safinamide is generally well-tolerated and safe, with few treatment-related mild adverse events.

Even if both preclinical and clinical studies suggest that safinamide does not induce significant modifications of the severity of L-DOPA-induced dyskinesia (Bette et al. 2018), preclinical studies demonstrated that safinamide induces a significant rescue of the above-mentioned glutamatergic biochemical and neurochemical correlates of dyskinesia (Gardoni et al. 2018; Morari et al. 2018). In particular, co-treatment of safinamide with L-DOPA prevented the striatal rearrangement of synaptic GluN2 subunits of the NMDA receptor and the rise in striatal glutamate associated with dyskinesia appearance (Gardoni et al. 2018; Morari et al. 2018).

10.5 Emerging Treatments Targeting Molecular Components of the Glutamatergic Synapse for Parkinson's Disease and L-DOPA-Induced Dyskinesia

10.5.1 Emerging Treatments Targeting NMDA Receptors

Early preclinical studies evaluated the efficacy of different types of NMDA receptor subunit-specific antagonists in improving motor behavior in experimental models of both Parkinson's disease and L-DOPA-induced dyskinesia (Loschmann et al. 2004; Nash et al. 2000; Wessell et al. 2004). The GluN2B-selective antagonists reduced parkinsonian symptoms in both rats and non-human primates models of Parkinson's disease (Nash et al. 2004; Morissette et al. 2006), but other reports described conflicting results about the efficacy of these compounds in reducing the onset of dyskinesia in experimental models (Wessell et al. 2004; Rylander et al. 2009). Unfortunately, a randomized, double-blind, placebo-controlled clinical trial showed that the GluN2B-antagonist CP-101,606 reduced the severity of L-DOPA-induced dyskinesia in patients, but it induced dose-related amnesia (Nutt et al. 2008). Only few studies have been performed on GluN2A-selective antagonists. Treatment with cell-permeable peptides disrupting GluN2A/PSD-MAGUKs interaction demonstrated that a decrease in synaptic GluN2A-containing NMDA receptors induces a significant improvement of motor behavior in parkinsonian rats (Paillé et al. 2010) and a reduction in the onset of dyskinesia (Mellone et al. 2015).

Other authors recently focused on the role of GluN2D-containing NMDA receptors mainly expressed in striatal ChIs and on the putative efficacy of newly

developed GluN2D-selective positive allosteric modulators (Feng et al. 2014). However, additional preclinical studies are still needed for a detailed comprehension of the role of the GluN2D in disease pathogenesis and before a putative clinical use of agents specifically targeting this NMDA receptor subunit.

Considering that the enrichment in specific regulatory GluN2-type subunits regulates NMDA receptor function and pharmacological properties (Paoletti et al. 2013), another putative therapeutic strategy could aim at restoring a physiological subunit composition synaptic content rather than acting on the receptor activity. The use of cell-permeable peptides disrupting GluN2A interaction with scaffolding proteins demonstrated that a decrease in synaptic GluN2A-containing NMDA receptor is sufficient to improve the motor behavior in parkinsonian rats (Paille et al. 2010) and reduces the onset and severity of established L-DOPA-induced dyskinesia in both rat and monkey models (Gardoni et al. 2012; Mellone et al. 2015; Stanic et al. 2017). In addition to NMDA receptor association with scaffolding proteins, also the D1-NMDA receptor heteromer has been recently considered a possible target to counteract L-DOPA-induced dyskinesia. Intrastriatal administration of a cell-permeable peptide disrupting D1-GluN1 binding reduced the severity of established dyskinesia (Song et al. 2016).

Finally, the noble gas xenon is capable of working as inhibitor of NMDA receptors (Haseneder et al. 2009), thus suggesting that also xenon could reverse L-DOPA induced dyskinesia. A recent preclinical study shows that xenon gas exposure normalized synaptic transmission and synaptic plasticity at corticostriatal glutamatergic projections, ameliorated dyskinesia in rat and non-human primate models, and improved gait performance in a non-human primate model of Parkinson's disease. Accordingly, these results pave the way for a future clinical testing of this unconventional approach (Baufreton et al. 2018).

10.5.2 Emerging Treatments Targeting AMPA Receptors

The putative activity in reducing dyskinesia of selective AMPA receptor antagonists in combination with L-DOPA therapy has been investigated in preclinical and clinical research. A competitive AMPA receptor antagonist reduced wearing-off of L-DOPA-induced motor responses in a rat model (Marin et al. 2001), while a non-competitive antagonist increased the anti-parkinsonian benefits of L-DOPA in a monkey model (Konitsiotis et al. 2000). However, clinical trials with AMPAR antagonists such as topiramate and perampanel, two drugs commonly used in the clinical practice for the treatment of epilepsy, have provided conflicting results (Eggert et al. 2010; Kobylecki et al. 2011; Kobylecki et al. 2014; Lees et al. 2012). Overall, other studies are warranted to clarify the efficacy of AMPA receptor compounds for the treatment of L-DOPA-induced dyskinesia.

10.5.3 Emerging Treatments Targeting mGluRs

Several preclinical reports have demonstrated the efficacy of selective mGluR modulators in the treatment of neurodegenerative disorders, including both Parkinson's disease (Rouse et al. 2000; Amalric 2015) and Alzheimer's disease.

In vivo treatment with mGluR4 agonists or positive allosteric modulators (PAM) produced beneficial effects in rodent models of Parkinson's disease (Valenti et al. 2003; Niswender et al. 2008; Beurrier et al. 2009). In addition, chronic treatment with mGluR4 agonist in combination with L-DOPA significantly reduced the development of abnormal involuntary movements. However, a single injection of the agonist was not sufficient to decrease the severity of already established dyskinesia (Lopez et al. 2011). Similarly, an mGlu4 receptor PAM combined with subthreshold doses of L-DOPA acts synergistically with the latter to alleviate akinesia and reduced the incidence but not the severity of dyskinesia (Bennouar et al. 2013).

A more recent study found that both mGluR4 orthosteric agonist and PAM did not alter the development of L-DOPA-induced dyskinesia and did not modify the abnormal involuntary movement score in animals with already established dyskinesia (Iderberg et al. 2015). However, mGluR4 PAM but not orthosteric agonist potentiated the motor stimulant effect of a subthreshold L-DOPA dose in specific behavioral tests (Iderberg et al. 2015), suggesting that a pharmacological stimulation of mGlu4 lacks intrinsic anti-dyskinetic activity, but it has a DOPA-sparing activity in the treatment of Parkinson's disease-associated motor symptoms. In addition, another study showed that co-treatment with selective mGluR4 PAM in association with a low dose of L-DOPA induces a robust dose-dependent reversal of the akinesia. Importantly, the enhancement of L-DOPA effect is not associated with a worsening of dyskinesia treated with the mGluR4 PAM (Le Poul et al. 2012).

Finally, the mGluR4 PAM PXT002331-foliglurax alleviated the motor symptoms of Parkinson's disease and the motor complications induced by L-DOPA in primates (Charvin et al. 2018).

All the above-mentioned preclinical results clearly support the putative therapeutic effect of the co-therapy with L-DOPA and an mGluR4 to maintain the benefits of L-DOPA on Parkinson' disease motor symptoms reducing the development of dyskinetic behavior. A recent phase 1 randomized, double-blind, placebo-controlled single and multiple ascending dose clinical trial has been successfully completed showing that the PXT002331-foliglurax (https://clinicaltrials.gov/ct2/show/NCT02639221) is safe and well-tolerated even at doses much higher than those necessary for robust effects in animal models of Parkinson's disease. A phase 2 trial to evaluate PXT002331-foliglurax efficacy, safety, and tolerability in reducing L-DOPA-induced motor complications is currently ongoing (https://clinicaltrials.gov/ct2/show/NCT03162874).

mGluR4 forms heteromeric complexes with other mGluR subtypes, such as mGluR2. These mGluR4/mGluR2 heteromers show distinct pharmacological profiles and different responses to allosteric modulators compared to mGluR4

homomers. In particular, some mGluR4 PAMs do not potentiate glutamate activity when mGluR2 and mGluR4 are co-expressed, whereas other compounds potentiate mGluR4 responses regardless of mGluR2 co-expression. Interestingly, a novel mGluR4 PAM (VU0418506) potentiates mGlu4 homomers in rodent models, but it fails to potentiate the activity of an mGluR4 agonist at mGluR2/4 heterodimers. These findings suggest that the anti-parkinsonian activity of mGluR4 PAMs is mediated by mGluR4 homomeric receptors without the involvement of mGluR2/4 heteromers (Engers et al. 2016; Niswender et al. 2016).

mGluR2 has been also involved recently in Parkinson's disease. mGluR2 regulates glutamate transmission and, upon activation, reduces glutamate release (Crupi et al. 2019). Consequently, mGlu2 stimulation could lead to a reduction of NMDA activation, thus suggesting a possible anti-dyskinetic effect. In agreement with this hypothesis, orthosteric activation of mGlu2/3 receptors reduces the severity of already established L-DOPA-induced dyskinesia in both the rat and the marmoset model (Frouni et al. 2019). In addition, a highly-selective mGluR2 PAM is effective in alleviating established and preventing the development of L-DOPA-induced dyskinesia in the rat model (Hamadjida et al. 2020) suggesting that mGluR2 activation may be an effective and promising therapeutic strategy to alleviate this L-DOPA-induced motor complication.

Modulation of mGluR5 activity has been considered for a long time an attractive pharmacological approach for the treatment of Parkinson's disease and L-DOPA-induced dyskinesia. mGluR5 antagonists or negative allosteric modulators (NAM) modulate the motor behavior in experimental models of L-DOPA-induced-dyskinesia (Fieblinger et al. 2014; Grégoire et al. 2011; Johnston et al. 2010; Morin et al. 2010; Rylander et al. 2010). Chronic treatment with the mGluR5 antagonist MPEP in the rodent model almost abolishes L-DOPA-induced dyskinesia (Levandis et al. 2008). Similarly, several studies confirmed the anti-dyskinetic properties of mGluR5 antagonists also in the monkey model (Grégoire et al. 2011; Johnston et al. 2010; Morin et al. 2010; Rylander et al. 2010). More recently, the mGluR5 NAM dipraglurant reduced both choreic and dystonic L-DOPA-induced dyskinesia in parkinsonian monkeys without inducing any modification of the efficacy of L-DOPA (Bezard et al. 2014). Overall these preclinical studies strongly suggested that mGluR5 modulators could be potentially useful for the treatment of L-DOPA-induced dyskinesia, thus supporting the evaluation of their efficacy in clinical trials. Early clinical studies suggested that the use of the mGluR5 NAM mavoglurant in combination with high doses of L-DOPA may be effective in treating Parkinson's disease patients with L-DOPA-associated motor fluctuations and dyskinesia. In particular, phase 2 trials investigated the safety and efficacy of mavoglurant and demonstrated the potential of this compound in reducing L-DOPA-induced-dyskinesia in patients (Berg et al. 2011). Concomitant administration of mavoglurant improved off-time in patients treated with high doses of L-DOPA without worsening their dyskinetic profile (Kumar et al. 2016). Unfortunately, this clinical study was limited by several key issues including the limited number of enrolled patients ($n = 7$/group), the short treatment duration, and the conflicting clinician-rated measures (Kumar et al. 2016). Conflicting results were obtained in two other

phase 2 randomized, double-blind studies which tested the effects of immediate-release (study 1) and modified-release (study 2) mavoglurant on LID. These studies report no significant improvement of L-DOPA-induced-dyskinesia and fail to replicate the previous outcome (Trenkwalder et al. 2016). In contrast, encouraging results were obtained in a recent phase 2 double-blind, placebo-controlled, randomized clinical trial where the NAM dipraglurant was tested in patients with moderate to severe L-DOPA-induced-dyskinesia. Moreover, dipraglurant was well-tolerated and induced a significant decrease of peak-dose dyskinesia on day 14 with no evidence of worsening parkinsonism (Tison et al. 2016). However, further studies in a larger cohort of patients are warranted to confirm the efficacy of dipraglurant in reversing L-DOPA-induced-dyskinesia.

A recent meta-analysis of 9 clinical trials evaluating the effects of mGluR5 antagonists for the treatment of patients with L-DOPA-induced dyskinesia did not recommend the use of mGluR5 antagonists for the routine treatment of patients right now (Wang et al. 2018). In fact, even if they found significant difference between mGluR5 antagonists and placebo in terms of abnormal involuntary movement score, there were no significant improvements in terms of LFADLDS (*Lang-Fahn Activities of Daily Living Dyskinesia scale*) and UPDRS Part IV (*Unified Parkinson's Disease Rating Scale*—motor complications). Moreover, adverse events incidence was higher with mGluR5 antagonists than with placebo, especially at the expense of increased dizziness, visual hallucination, or fatigue (Wang et al. 2018).

10.6 Conclusion and Future Perspectives

Molecular and functional interactions between dopamine and glutamate regulate a high variety of brain functions such as motor control, cognition, and memory and many others and, when altered, they play an important role in numerous central nervous system disorders. Several studies in the last twenty years, described in this chapter, focused on the understanding of the mechanisms coordinating the cross-talk between glutamate and dopamine and its relevance in Parkinson's disease. Hopefully, a complete knowledge of the dysregulation between glutamate and dopamine signaling could represent the first step for the identification and setting up of novel therapeutical approaches for this brain disorder (see Fig. 10.1).

The pathophysiological picture emerging from all the above-mentioned studies shows that the strength of glutamatergic signals from the cortex to the striatum is dynamically regulated during the progression of Parkinson's disease and following chronic dopaminergic therapy with L-DOPA. Accordingly, the complete characterization of the role played by the glutamatergic synapse in Parkinson's disease-associated motor alterations is essential for a full comprehension of disease pathogenesis and the setting up of a novel pharmacological intervention strategy targeting glutamate neurotransmission. The efficacy of glutamate receptors to modulate synaptic transmission in the striatum indicates that modulation of the activity of

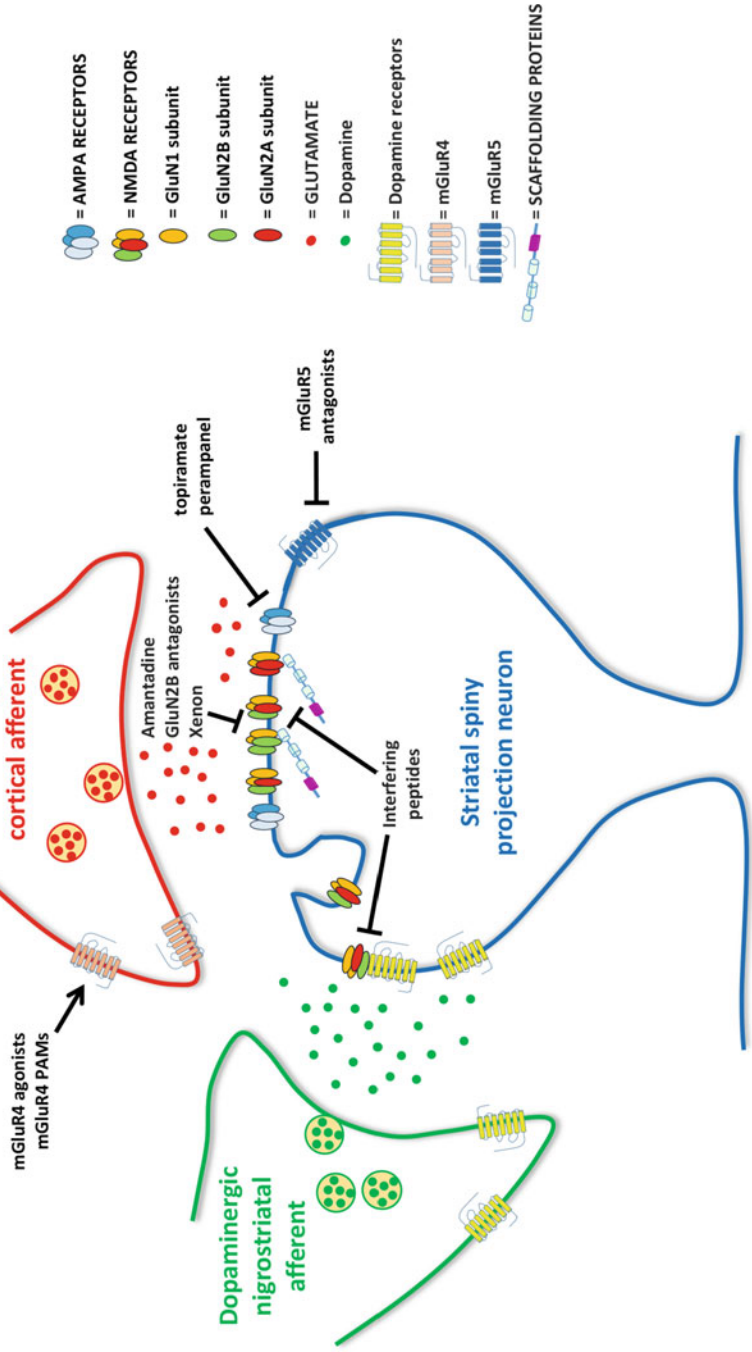

Fig. 10.1 Main compounds evaluated in preclinical and clinical settings targeting pre- and postsynaptic glutamate receptors for therapeutic intervention in PD

these receptors may represent a key target to rescue the altered neurotransmission in Parkinson's disease (see Fig. 10.1).

At present, two drugs targeting the glutamatergic synapse are used in the clinical practice as add-on treatment to L-DOPA for the management of motor fluctuations (safinamide) or to decrease the severity of dyskinesia (amantadine). As described above, safinamide reduces glutamate release from presynaptic terminals while amantadine mainly acts as low-affinity antagonist of NMDA receptor. Taking into account results obtained on the molecular and functional alterations of ionotropic and metabotropic glutamate receptors in experimental models of Parkinson's disease and L-DOPA-induced dyskinesia, several preclinical and clinical studies have been performed by using different types of receptor modulators. Unfortunately, in many cases encouraging results described in animal models have not been fully confirmed in clinical trials, even if the role played by NMDA receptors and mGluRs in disease pathogenesis and in dyskinesia has been clearly addressed and confirmed. However, some disappointing results obtained in clinical trials with agents modulating mGluRs suggest that a lot of effort is still required to understand the molecular basis of dyskinesia and to develop effective therapeutic strategies able to halt the motor abnormalities which affect parkinsonian patients after prolonged administration of L-DOPA.

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Suggested Reading

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Chapter 11
The Modulation of Glutamatergic Signaling as a Potential Therapeutic Strategy for Major Depression

Patricio Molero, Enrique Aubá, María del Mar Unceta, and Felipe Ortuño Sánchez-Pedreño

Abstract The aim of this chapter is to provide an update of the most relevant advances in the conceptualization of the modulation of glutamatergic signaling as a potential therapeutic strategy for Major Depressive Disorder (MDD), from animal models to recent phase III clinical trials. The main clinical features of MDD, its epidemiological impact in terms of disease burden, its mainstream pharmacological treatment, and current unmet needs are presented. A brief review of the implications of the glutamatergic system for MDD follows, together with its close relationship with the gabaergic system. The main molecular components of the glutamatergic system as potential drug targets for MDD are presented, namely selective NMDA receptors (NMDAR) antagonism, AMPA receptors (AMPAR) potentiation, metabotropic glutamate receptors 2 and 3 (mGluR2 and 3) antagonism, excitatory amino acid transporters (EAAT) potentiation, mammalian target of the rapamycin complex 1 (mTORC1) modulation and GABA-A receptor modulation. The current modulators of the glutamatergic signaling for the treatment of MDD are presented: intravenous ketamine (off-label use for MDD), intranasal esketamine (FDA approved for TRD), and intravenous brexanolone (FDA approved for postpartum depression). In addition, some emerging treatments related to glutamate modulating drugs for MDD are mentioned with their rationale (R-ketamine, rapastinel, lanicemine, and other compounds).

Keywords Major depressive disorder · Treatment-resistant depression · Glutamate · GABA · Ketamine · Esketamine

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_11

Abbreviations

AEs	Adverse events
AMPAR	α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor
BDNF	Brain-derived-neurotrophic-factor
EAAT	Excitatory amino acid transporters
eEF2	Eukaryotic elongation factor 2
GABA	Gamma-aminobutyric acid
GABA-R	GABA receptor
Glu	Glutamate
HADRS	Hamilton depression rating scale
HNK	Hydroxynorketamine
i.v.	Intravenous
MADRS	Montgomery-Asberg depression rating scale
MDD	Major depressive disorder
mGluR2 and 3	Group II metabotropic glutamate receptors 2 and 3
mPFC	Medial PFC
mTORC1	Mammalian target of rapamycin complex 1
NMDAR	*N*-methyl-D-aspartate (NMDA) receptor
NR2B	NMDA receptor subunit 2B
PET	Positron emission tomography
PFC	Prefrontal cortex
R-HNK	R-hydroxynorketamine
SNRI	Serotonin-norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
TRD	Treatment-resistant depression
TrKB	Tropomyosin receptor kinase B

11.1 Introduction: Major Depressive Disorder

Major depressive disorder (MDD) is a persistent state (of at least 2 weeks) defined clinically as a combination of either persistent depressed mood and/or loss of interest (apathy) or pleasure (anhedonia), together with other symptoms such as fatigue or loss of energy, psychomotor retardation or agitation, reduced concentration and attention (with diminished ability to think and indecisiveness), reduced self-esteem and self-confidence, ideas of excessive or inappropriate guilt and worthlessness, hopelessness, disturbed sleep (insomnia or hypersomnia), reduced appetite with weight loss (or increased appetite). It may be associated with suicidal thoughts (ranging from recurrent thoughts of death to suicide ideation with a plan or suicide attempt in severe forms) or with delusional ideas in severe forms. These clinical features cause a significant impairment of the global functioning (in the

socio-familiar and occupational spheres) and important distress and suffering for the person presenting them and their relatives and significant others (World Health Organization 2004; American Psychiatric Association 2013).

From the epidemiological point of view, MDD is highly prevalent in western society, and this translates into a serious public health problem of growing concern: it was the fourth leading cause for the burden of disease already in 1990 (Murray and Lopez 1997), remains as one of the most important causes of Years Lived with Disability (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators et al. 2017) and furthermore it is expected to be the leading cause of disease burden by 2030 (Lépine and Briley 2011).

The treatment of MDD consists of a combination of psychotherapy (usually for mild and moderate forms) and psychopharmacological treatment (indicated in moderate and severe forms). Another therapeutic modality is based on neurostimulation treatments, mainly electroconvulsive therapy (which requires general anesthesia and is reserved for severe or refractory forms or in situations which require a fast response or when a proper psychopharmacological treatment is not possible, due to comorbidities or other situations such as pregnancy).

Pharmacological treatment is essential in the therapeutics of MDD and during the last 60 years, the mainstream pharmacodynamic strategy for the majority of antidepressant drugs has been the modulation of monoamine neurotransmission systems, namely acting on the selective transporters of serotonin (SERT), noradrenaline (NET) and dopamine (DAT) to block reuptake, and on monoamine oxidase enzyme to inhibit monoamine catabolism. Although these drugs originate immediate neurobiological, post-receptorial effects (e.g., upon neuronal metabolism and genetic expression processes), the clinical therapeutic effect typically appears after 3–6 weeks of the initiation of treatment. This period can be problematic when a fast response is needed (as in cases of suicide risk or delusional depressive ideation). Also, the global effectiveness of this scheme is not optimal, with estimations of a global remission rate in the order of 30% after 12 weeks of treatment and in the order of 70% after four sequential treatments (Rush et al. 2006).

Although there is not a consensus for the definition of treatment-resistant depression, a proposed definition is the failure to achieve remission with two or more adequate antidepressant trials, and there are several staging models for a progressively increased resistance, from one to several antidepressant drugs, of the same or different class, or inclusion of electroconvulsive therapy (McIntyre et al. 2014). The prevalence of TRD has been estimated in 12-month prevalence rates of about 3% for Stage 1 TRD (failure to respond to 1 adequate trial of an antidepressant) and about 2% for Stage 2 TRD (failure to respond to 2 adequate trials) (Nemeroff 2007).

With these figures, it becomes clear that the improvement of both the long latency of the therapeutic clinical response to available antidepressants and the low remission rates are important unmet needs in the treatment of MDD.

11.2 The Glutamatergic System in Mood Disorders

Glutamate (Glu) is the main excitatory neurotransmitter in the mammalian brain (Orrego and Villanueva 1993) and is involved in the regulation of emotional and cognitive processes: it has a prominent role in synaptic plasticity, learning and memory, but in pathological conditions it is a potent neuronal excitotoxin, triggering either fast or delayed neurotoxicity (Sanacora et al. 2008). Excessive exposure to Glu or a hyperstimulation of Glu receptors causes a detrimental process called excitotoxicity, which causes neuronal death or dysfunction. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammal brain, and both systems are reciprocally regulated to maintain a balance of excitation/inhibition (Schoepp 2001). Cortical activity is the result of a balance between excitation and inhibition, and the activity of both systems is closely related: Glu is the metabolic precursor of GABA, which can be recycled through the tricarboxylic acid cycle to synthesize glutamate (Petroff 2002).

Glutamate is synthesized from glucose or through the glutamate/glutamine cycle, is present in a dense network of both neurons and glial cells (astrocytes) (Erecińska and Silver 1990) and is transported into synaptic vesicles where it is stored until its release to the synaptic cleft by exocytosis (Sanacora et al. 2008). After release, Glu binds to glutamate receptors throughout the brain. Clearance of glutamate from the extracellular space through the excitatory amino acid transporters 1 to 5 (EAAT1-5) is essential to avoid excitotoxicity, given the lack of Glu catabolic enzymes in the synapse. This precisely is one of the most important functions of astrocytes that express EAAT1 and EAAT2 (EAAT3-5 are exclusively neuronal) (Murphy-Royal et al. 2017).

There are two main types of glutamate receptors: ionotropic (ligand-gated ion channels: AMPA receptors and Kainate receptors, that mediate the initial response to Glu by allowing inward flow of Na+, and NMDA receptors (NMDAR), blocked by Mg+ at rest) and metabotropic (group I, acting through PLCβ and activation of IP3 and DAG -mGluR1a-d and mGlu5a-b-, and groups II -mGluR2 and 3- and III -mGluR4 and mGluR6-8- acting through negative coupling to adenylyl cyclase) (Sanacora et al. 2008).

As mentioned above, moderate levels of NMDAR activation promote neuroprotective signaling pathways, including activation of the RAS–mitogen-activated protein kinase (RAS–MAPK) pathway and cyclic AMP-responsive element-binding protein (CREB)-mediated expression of brain-derived neurotrophic factor (BDNF), with a key role in neuroprotective and neurotrophic processes that are relevant to stress and mood disorders (Murrough et al. 2017). However, overactivation of NMDAR leads to excitotoxicity that contributes to the pathogenesis of neurodegenerative diseases (Dong et al. 2009) including Parkinson's disease (Kaur et al. 2019), Alzheimer's disease (Wang and Reddy 2017), and Huntington disease (Fan and Raymond 2007).

An abnormal glutamatergic activity has been proposed as a contributing factor to the impairments in synaptic and neural plasticity of severe or recurrent mood

disorders (Sanacora et al. 2008). In a comprehensive review of this matter, Sanacora and colleagues from Yale University and NIMH-NIH (USA) articulate the evidence on glutamate neurotransmission and mood disorders in these important domains: (1) changes in glutamate levels of plasma, serum, cerebrospinal fluid, and brain tissue; (2) Glutamate receptor alterations, including differences related to NMDAR expression and binding affinities between individuals with and without mood disorders, and genetic polymorphisms of the GRIN1 gene coding for the NR1 subunit and the GRIN2B gene coding for NR2B; (3) Evidence of glial-cell pathology, namely reduced numbers of oligodendrocytes and reduced expression of excitatory amino acid transporters (EAATs), which clear glutamate from the synaptic cleft.

Another aspect of the possible implication of the glutamatergic system in MDD is related with the relationship between chronic stress, chronic depressive symptoms and neuronal atrophy in the prefrontal cortex (PFC) and hippocampus, which may be mediated by the glutamatergic activity in a model extensively reviewed by Murrough et al. (Murrough et al. 2017): acute stress increases glutamate release, and chronic stress induces a reduction in synaptic AMPAR and NMDAR availability in the cortex and maladaptive changes within glutamate synapses, including reduced extracellular glutamate clearance by glia and the increased activation of extrasynaptic NR2B-containing NMDARs, potentially contributing to synaptic loss and the activation of cellular apoptotic pathways, all this resulting in a decrease in synaptic functioning and contributing to features of MDD as changes in glutamate levels, reductions in brain volume and altered function and connectivity within brain networks that are crucial for mood regulation.

In order to understand the relevance of the glutamatergic system in MD, it is important to conceptualize it in the functional context of the known neurobiological underpinnings of depression, especially regarding the monoaminergic system and the Glutamate-GABA balance.

There is evidence supporting an important relationship between the glutamatergic and the serotonergic systems regarding the antidepressant effect (a review of this matter can be found in Chaki and Fukumoto (2019). mGluR2 and 3 antagonism exert antidepressant effects in rodent models similar to those of ketamine, with shared synaptic response and neural mechanisms and, interestingly, an implication of the serotonergic system (Chaki and Fukumoto 2019): both ketamine and mGluR2 and 3 receptor antagonists increase the 5-HT extracellular levels in the rat medial prefrontal cortex (mPFC) through activation of the AMPA receptor (which leads to an increase in activity of 5-HT neurons in the dorsal raphe nucleus (DRN), presumably via the mPFC-DRN projection), and the antidepressant actions of ketamine in the Forced Swim Test (FST) are blocked under pharmacological depletion of 5-HT in the brain with pretreatment with para-chlorophenylalanine (PCPA), an irreversible inhibitor of tryptophan hydroxylase (Gigliucci et al. 2013). These data suggest that the AMPA receptor-dependent 5-HT release in the mPFC may be closely involved in the antidepressant effects of the mGlu2/3 receptor antagonist and ketamine (Chaki and Fukumoto 2019).

Precisely the evidence of the anatomical connection and reciprocal regulation between prefrontal cortex (PFC) and dorsal raphe (DR)—of GABA interneurons and

glutamatergic pyramidal neurons of PFC (by serotoninergic projections from DR) and DR serotoninergic neurons (by PFC pyramidal neurons) (Warden et al. 2012)—has allowed a hypothesis of monoamine (5-HT)–Glutamate/GABA long neural circuit proposed by Yun-Fen Li from the Beijing Institute of Pharmacology and Toxicology (Li 2020). According to this model, the serotoninergic activity of DR is regulated by long projection from pyramidal neurons of PFC (through AMPA receptors) and by nearby GABA interneurons (through regulation of GABA-A receptor); and in PFC, the excitatory/Inhibitory balance of glutamatergic pyramidal neurons and GABA interneurons, respectively, is regulated by long-projections of serotoninergic neurons from DR (via 5-HT receptor such as 5-HT1AR). In this model, the excitatory/Inhibitory (Glu/GABA) rebalance is the rate-limiting step for the onset speed of antidepressant, and rapid activation of this circuit would allow the PFC to rapidly reach the E/I balance, and rapidly enhance synaptic plasticity by BDNF-mTOR pathway (Li 2020).

11.3 Molecular Components of the Glutamate Neurotransmitter System as Potential Drug Targets for MDD

11.3.1 Selective NMDAR Antagonism

The evidence of the clinical effects of a single subanesthetic dose of ketamine, a NMDAR antagonist, since the seminal trial of Berman and colleagues from Yale University in the year 2000 (Berman et al. 2000) (namely, a rapid, sustained though transient—from 72 h to 1 week—and a short psychotomimetic and cardiovascular effects immediately postdose) has led to the investigation of the precise nature of NMDAR antagonism (in terms of anatomical connection and intensity) that yields an antidepressant mechanism, given that other NMDAR antagonists do not produce antidepressant effects.

The functional antagonism of NMDAR of the GABA inhibitory neurons has been postulated as the mechanism underlying the dissociative effects of ketamine (Homayoun and Moghaddam 2007). Ketamine antagonism of NMDAR deactivates eukaryotic elongation factor 2 (eEF2) kinase (reduced eEF2 phosphorylation) leading to de-suppression of translation of BDNF (Autry et al. 2011). It has been postulated that the antidepressant effect is mediated by the antagonism of extrasynaptic NMDAR subtype expressing the NMDA receptor subunit 2B (NR2B) (Lang et al. 2017), de-suppressing protein synthesis and inducing antidepressant actions via an mTOR-dependent mechanism (Zanos and Gould 2018). A long-lasting activation of BDNF–TrkB cascade in the PFC and hippocampus might be implicated in the long-lasting antidepressant effects of ketamine and its enantiomers (Hashimoto 2019).

The evidence from mice models of depression suggests that the antidepressant effect of ketamine might not necessarily be dependent upon NMDAR antagonism. Indeed, Zanos et al. demonstrated that: (1) the ketamine metabolite R-hydroxynorketamine (R-HNK) exerts behavioral, electroencephalographic, electrophysiological, and cellular antidepressant actions that are independent of NMDAR inhibition, but involve activation of AMPARs; and (2) R-HNK lacks ketamine related side effect (Zanos et al. 2016). However, this evidence needs to be replicated and translated to clinical research. On the other hand, interestingly, a significant association has been found between increased dissociative symptoms (ketamine's side effects) at 40 min postdose and percent improvement with ketamine in depressive symptoms in the Hamilton Depression Rating Scale (HDRS) at 230 min and Day 7, which raises the hypothesis of whether the dissociative side effects of ketamine mediate its antidepressant effects (Luckenbaugh et al. 2014).

The glycine site of NMDARs is a promising target for antidepressant and procognitive effects, and indeed six glycine site modulators with procognitive and antidepressant properties have been identified (D-serine (co-agonist), D-cycloserine (partial agonist), D-alanine (co-agonist), glycine (agonist), sarcosine (co-agonist), and rapastinel (partial agonist) (Peyrovian et al. 2019).

A very intriguing, recent set of findings raises the question of a possible antidepressant effect of circulating autoantibodies (IgM, IgA, and IgG) against NMDAR subunit NR1, that are endogenously synthesized in some pathological conditions and stress (Pan et al. 2021). If confirmed in translational experimentation, this may represent a novel opportunity to apply the principles of immunotherapy to major depression.

11.3.2 AMPA Receptors Potentiation

It has been hypothesized that the rapid antidepressant effect of NMDAR antagonism might require AMPA receptors activation, given that pretreatment with an AMPAR antagonist attenuates the ketamine-induced antidepressant-like behavior in mice (Maeng et al. 2008). It has been proposed that ketamine has a unique ability to increase the AMPA–NMDA receptor throughput by directly blocking NMDA receptors and indirectly enhancing AMPAR density and/or function, which in turn activates downstream synaptogenic signaling pathways (e.g., BDNF, mTOR) (Aleksandrova et al. 2017).

11.3.3 mGluR2 and 3 Antagonism

These metabotropic receptors are highly expressed in regions associated with cognition and emotion (cortical and limbic areas). mGluR2 knockout mice show antidepressant-like behavior in the forced-swim test and an enhanced rewarding

responsiveness to cocaine (with increased release of DA and Glu in Nucleus Accumbens (NAc) (Morishima et al. 2005). mGlu2/3-R antagonism exerts antidepressant effects in rodent models similar to those of ketamine, with shared synaptic response and neural mechanisms, which implies the serotonergic system (Chaki and Fukumoto 2019). Indeed, serotonergic transmission plays critical roles in the antidepressant effects of both mGluR2/3 receptor antagonists and ketamine, that has been proposed to be mediated by 5-HT1A in the medial PFC (Chaki and Fukumoto 2019).

11.3.4 EAAT Potentiation

A potential mechanism of neuroprotection from excitotoxicity is to enhance glutamate reuptake, and this mechanism may be relevant for novel glutamatergic modulators with antidepressant activity. Indeed, decreased expression of EAATs has been observed in postmortem studies of subjects with mood disorders, whereas an increased expression (e.g., as induced by ß-lactam antibiotics) may induce antidepressant-like effects (Zarate et al. 2010). A potentiation of EEAT activity has been suggested as a potential antidepressant mechanism (Lapidus et al. 2013). Moreover, Colton and colleagues from the Ohio State University have identified a series of compounds that can induce translation of EAAT2 transcripts (Colton et al. 2010), and this may represent the first step for the preclinical investigation of this mechanism.

11.3.5 Mammalian Target of the Rapamycin Complex 1 (mTORC1) Modulation

Mammalian target of rapamycin (mTOR) is a protein kinase involved in translation control and long-lasting synaptic plasticity, and consists of two multi-protein signaling complexes, mTORC1 and mTORC2 (Hoeffer and Klann 2010). Li et al. from Yale University demonstrated that ketamine produces a fast activation of the mTOR pathway, leading to increased synaptic signaling proteins and increased number and function of new spine synapses in the prefrontal cortex of rats, and that blockade of mTOR signaling blocks both ketamine's antidepressant effects and induced synaptogenesis (Li et al. 2010). In agreement with the glutamate/GABA regulation hypothesis described above (Li 2020), there is evidence of the requirement of GABA-B receptors signaling for the mTORC1-dependent protein synthesis underlying rapid antidepressant effect (Workman et al. 2013). However, the role of mTORC1 as a mediator of antidepressant effects is not clear given that in a clinical trial in TRD, the mTORC1 inhibitor rapamycin not only did not suppress the

antidepressant activity of ketamine but it tripled its antidepressant effect (Abdallah et al. 2018).

11.3.6 GABA-A Receptor Modulation

Positive and negative allosteric modulators of a GABA-A receptor expressed preferentially on the hippocampus (α5-GABA-A receptor) have shown sustained antidepressant-like effects in rodents in the absence of abuse-like properties, restoration of synaptic function and behavioral deficits provoked by chronic stress and anxiolytic-like effects, putatively via an increased signal-to-noise ratio of hippocampal transmission (decreasing the tonic hippocampal activity without dramatically altering phasic activation of pyramidal neurons) (Carreno et al. 2020). This mechanism may represent another opportunity for novel glutamatergic modulators with antidepressant activity.

11.4 Current Modulators of the Glutamatergic Signaling for the Treatment of MDD

11.4.1 Ketamine and Esketamine

Beyond unspecific NMDARs antagonism, proposed mechanisms of ketamine's antidepressant action have been reviewed extensively by Panos Zanos and Todd D. Gould from the University of Maryland School of Medicine (Zanos and Gould 2018). In their review, this complex mechanism of action is based on several domains, including: (1) GluN2B-selective extrasynaptic NMDAR inhibition and inhibition of NMDARs of GABAergic interneurons (disinhibition hypothesis, leading to pyramidal cell disinhibition and an enhancement of excitatory glutamatergic neurotransmission in the medial prefrontal cortex) and (2) inhibition of NMDAR-dependent burst firing of lateral habenula neurons (with supporting preliminary evidence from a PET study (Carlson et al. 2013)); together with downstream effects upon synaptic plasticity, namely: (3) α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (AMPARs) activation; (4) phosphorylation (activation) of tropomyosin receptor kinase B (TrKB) and increased expression of brain-derived neurotrophic factor (BDNF); (5) inhibition of the phosphorylation of the eukaryotic elongation factor 2 (eEF2) kinase; and (6) activation of the mammalian target of rapamycin (mTOR) signaling. There is also evidence from mice models of depression of the contribution of the ketamine metabolite R-hydroxynorketamine (R-HNK) through mechanisms independent from NMDAR antagonism (Zanos et al. 2016).

In 2000, Berman et al. demonstrated, in the first randomized, double-blinded clinical study specifically designed to determine the antidepressant effects of

ketamine, that a single dose of intravenous (i.v.) ketamine 0.5 mg/kg monotherapy yielded a significantly greater reduction in depressive symptoms (as measured with the Hamilton Depression Rating Scale [HDRS]) than saline treatment after 72 h, in seven patients with MDD (Berman et al. 2000). This finding inspired a series of Single-Dose, Proof-of-Concept Studies with ketamine in the following years that globally assessed responses to a single dose of intravenous ketamine in over 150 patients with treatment-resistant depression (TDR) with multiple treatment failures, including electroconvulsive therapy (ECT), with evidence of a very fast improvement in depressive symptoms (within hours), with a response rate over 60% in the first 4.5 and 24 h, and over 40% after 7 days, with a big effect size in comparison with placebo (Cohen's d 1.3–1.7) or active placebo (midazolam, $d = 0.8$) (a review of the clinical efficacy can be found in Molero et al. (2018)). These figures contrast with the average effect size of monoaminergic antidepressants (Cohen's d 0.53–0.81) (Fournier et al. 2010) and their response latency (about 4–7 weeks) (Rush et al. 2006). After negative results of trials aimed to augment or prolong this antidepressant effect of ketamine with a different known glutamatergic modulator approved for clinical practice (riluzole) (Mathew et al. 2010; Ibrahim et al. 2012), another wave of clinical trials of repeated doses of i.v. ketamine monotherapy 0.5 mg/kg was conducted. Overall, these trials provided evidence that repeated doses (two to three doses per week) maintain the response over several weeks (18 (Murrough et al. 2013) to 28 (Shiroma et al. 2014) days), with an optimal dose frequency of twice-weekly regimen (rather than a thrice-weekly regimen) (Singh et al. 2016b).

In addition to the antidepressant effect, i.v. ketamine (0.5 mg/kg over 40 min) has been associated with a reduction in suicidality 24 h after a single dose (monotherapy) in patients with TRD, and this effect can be sustained for 12 days with repeated doses (Price et al. 2009). There is evidence suggesting that this antisuicidal effect is partially independent of the antidepressant effect (Grunebaum et al. 2018) and may be mediated by a reduction of nocturnal wakefulness (Vande Voort et al. 2016).

The S-enantiomer of racemic ketamine (esketamine) has higher affinity for the NMDARs than the R-enantiomer and exhibits a two to four times more potent antagonism (Oye et al. 1992; Zeilhofer et al. 1992; Moaddel et al. 2013). An optimal risk/benefit ratio of intravenous esketamine for TRD was found for a low dose (0.20 mg/kg over 40 min) with similar response rates than intravenous ketamine (over 60%) and better tolerability profile (Singh et al. 2016a). The advantages of the intranasal route regarding safety (compared with intravenous route) and bioavailability (compared with oral route) led to pilot trials with intranasal esketamine for MDD and TRD, with efficacy results comparable to intravenous ketamine 0,5 mg/kg (Lapidus et al. 2014; Daly et al. 2017).

Two recent phase 3, double-blind, active-controlled, multicenter randomized clinical trials have supported the efficacy and safety of esketamine nasal spray as a rapidly acting antidepressant for patients with treatment-resistant depression, as an add-on treatment combined with an oral conventional antidepressant (serotonin-norepinephrine reuptake inhibitor—SNRI—or selective serotonin reuptake inhibitor—SSRI) (Daly et al. 2017; Popova et al. 2019). In the first one, 197 patients

completed a 28-day intensive, induction treatment phase (56 or 84 mg of intranasal esketamine twice weekly during 4 weeks), yielding significantly greater improvement in the Montgomery-Asberg Depression Rating Scale (MADRS) score with esketamine plus antidepressant than with antidepressant plus placebo at day 28, with clinically meaningful improvement observed in the esketamine plus antidepressant arm at earlier time points also (days 2 (24 h), 8 and 22 after first dose of esketamine) (Popova et al. 2019). The response and remission rates in the esketamine arm in this study were 69,3% (with an NNT of 6) and 52,5% (with an NNT of 5), respectively. In order to estimate the clinical relevance of these figures, these response and remission rates were higher than those reported in the STAR*D trial for step 3 (16.8% and 13.7%, respectively) (Rush et al. 2006), and the only medication approved for the treatment of TRD at that time (olanzapine plus fluoxetine) has an NNT of 8 and 13, respectively (Citrome 2010).

The second one was designed to assess the efficacy of esketamine nasal spray in delaying relapse of depressive symptoms. This study demonstrated that in patients with TRD who experienced stable remission or response (176 and 121 patients, respectively) after 16 weeks of initial esketamine treatment, continuation of esketamine during a period of up to 88 weeks (either once weekly or every 2 weeks depending on an algorithm based on MADRS score) in addition to oral antidepressant treatment resulted in clinically meaningful superiority in delaying relapse compared with antidepressant plus placebo (Daly et al. 2017). Compared with antidepressant plus placebo treatment, esketamine plus antidepressant treatment decreased the risk of relapse by 51% among patients who achieved stable remission and 70% among those who achieved stable response.

Tolerability of i.v. ketamine (0.5 mg/kg over 40 min) seems acceptable in the short term, with postdose transient hemodynamic changes in about 30% of patients (increases in pulse and mean blood pressure, with a mean systolic increase of 19.6 $\pm$ 12.8 mmHg and a mean diastolic increase of 13.4 $\pm$ 9.8 mmHg) and a profile of common, reversible non-vital-risk-associated effects (drowsiness, dizziness, poor coordination, blurred vision, dissociative and psychotomimetic effects like feeling strange or unreal), peaking 40 min postdose, prolonging during the first 2 h postdose and generally resolving by 4–24 h (Wan et al. 2015).

As expected, intranasal esketamine offers an improved tolerability profile. The five most common frequent adverse events (AEs) in the intensive, induction phase were dissociation, nausea, vertigo, dysgeusia, and dizziness. These AE generally appeared shortly after dosing and resolved by 1.5 h after dosing. Also, transient blood pressure increases occurred after each dose of esketamine; the maximum value was reached at 40 min after dosing in most cases (mean maximum increases of 11.6 mmHg systolic, and 8.1 mmHg diastolic) and typically returned to or near the predose range by 1.5–2 h after dosing (Popova et al. 2019). In the maintenance phase, the five most common frequent AEs were dysgeusia, vertigo, dissociation, somnolence, and dizziness. Most AEs were mild to moderate, observed after dosing, and generally resolved in the same day. Importantly, no cases of respiratory depression or interstitial cystitis were observed (Daly et al. 2017). In an evaluation of the cardiovascular safety of esketamine nasal spray, combined with an oral

antidepressant, including 1708 esketamine-treated adults with TRD in six trials, of 4–52 weeks' duration, blood pressure elevations following esketamine dosing were not associated with clinically relevant changes of ECG parameters, are generally transient, asymptomatic, and not associated with serious cardiovascular safety sequelae (Doherty et al. 2020).

All in all, this evidence suggests that esketamine nasal spray (either 56 or 84 mg) plus a newly initiated oral antidepressant (SSRI or NSRI) demonstrated: (1) a clinically meaningful and statistically significant improvement in depressive symptoms compared to treatment with a newly initiated oral antidepressant treatment plus placebo nasal spray (as assessed by change in MADRS total score after 28 days in adult patients with TRD and in terms of time to relapse after 16 weeks of treatment); (2) a positive benefit-risk assessment of esketamine nasal spray as a novel treatment for patients with TRD. However, the generalizability of the study findings may be limited by the exclusion of participants with significant psychiatric or medical comorbidities or substance dependence.

Ketamine use for MDD remains an off-label indication. The American Psychiatric Association (APA) Council of Research Task Force on Novel Biomarkers and Treatments has published a consensus statement on this use with useful and detailed recommendations on key aspects such as patient selection, clinician experience and training, treatment setting, medication delivery, and follow-up and assessments (Sanacora et al. 2017a).

On March 5, 2019, the FDA approved the first ketamine product (esketamine, nasal spray) for treatment-resistant depression (indicated in conjunction with an oral antidepressant—not as monotherapy—for the treatment of TRD in adults) (FDA 2019a). Major concerns remain in terms of establishing an effective protocol to maintain the clinical antidepressant effect of ketamine/esketamine seen with acute administration while managing long-term safety, specifically regarding the potential for neurocognitive and urologic toxicity and the induction of substance use disorders (Molero et al. 2018). Thus, evidence from common clinical practice still needed to establish conclusions that are more definite.

11.4.2 Brexanolone

An exogenous analog of allopregnanolone, it acts as a positive allosteric modulator at the GABA-A receptor (Schumacher et al. 2014), and therefore may modulate the glutamatergic system to reestablish the Excitatory/Inhibitory (E/I) (Glu/GABA) balance in MDD to achieve a fast antidepressant response according to the aforementioned Li's model (Li 2020). Allopregnanolone levels raise progressively during pregnancy and rapidly fall in the postpartum period, which may participate in the genesis of postpartum depression (Nappi et al. 2001). A single i.v. administration of brexanolone during 60 h has demonstrated an antidepressant effect in women with severe postpartum depression in the 24 h timepoint, maintained during 30 days (Kanes et al. 2017). Brexanolone is the first Food and Drug Administration

(FDA)-approved treatment for moderate to severe postpartum depression in adults (FDA 2019b). The authorized administration consists of a slow, 60 h intravenous drip under continuous monitorization of a healthcare professional, and most common side effects are sedation/somnolence/dizziness, dry mouth, loss of consciousness, and flushing/hot flush (FDA 2019c).

11.5 Emerging Treatments Related to Glutamate Modulating Drugs for MDD

The discovery of the antidepressant efficacy of ketamine and its clinical development has inspired significant research efforts focused on the search of new molecules with a pharmacodynamics based on the modulation of the glutamatergic system, aiming for a fast antidepressant effect without the adverse reactions and inherent disadvantages of ketamine.

11.5.1 R-Ketamine

In 2014, Hashimoto and colleagues from Chiba University (Japan) demonstrated that (R)-ketamine produced longer-lasting antidepressant actions than (S)-ketamine in mice after neonatal dexamethasone exposure (Zhang et al. 2014), apparently without psychotomimetic side effects and abuse liability (Yang et al. 2015). The tolerability of R-Ketamine may be better than S-Ketamine according to limited human preclinical research. An investigation of the differential psychopathology and patterns of cerebral glucose metabolization caused by (S)- and (R)-ketamine in healthy volunteers using positron emission tomography (PET), with an i.v. infusion of a subanesthetic dose of both isomers (0.014–0.02 mg/kg/min over 53 min), yielded that equimolar doses of (R)-ketamine—in contrast to (S)-ketamine—did not produce psychotomimetic symptoms, but a state of relaxation and a feeling of well-being. Some of the subjects described their (R)-ketamine experience as a state of facilitated introspection comparable to a meditative state. (S)-ketamine-induced metabolic hyperfrontality as well as the metabolic changes in the left temporomedial and lateral cortex, basal ganglia, and occipital cortex. By contrast, (R)-ketamine produced opposite effects on cerebral metabolic rates of glucose (Vollenweider et al. 1997). A clinical trial of (R)-ketamine in depressed patients is currently ongoing (Hashimoto 2019).

11.5.2 Rapastinel

Rapastinel (previously GLYX-13) is an amidated tetrapeptide, generated from an amino acid sequence obtained from a hypervariable region of the light chain of a monoclonal antibody with NMDAR-modulating properties, which acts as a partial agonist at the glycine site of the NMDAR (Zhang et al. 2008). This positive allosteric modulation of NMDA receptors produces convergent effects with ketamine, including increased synaptic function and reverses the deficits caused by chronic stress exposure through effects on BDNF and mTORC1, which may translate into rapid and sustained antidepressant actions (Kato and Duman 2020). Indeed, at least nine clinical trials have been initiated of Rapastinel for MDD, with evidence of rapid antidepressant effects and beneficial effects on cognition and suicidality, without psychotomimetic side effects (Ragguett et al. 2019). Rapastinel has potential to produce procognitive effects, especially for age-associated cognitive impairment (Kato and Duman 2020). The results of a proof-of-concept, double-blind, randomized, placebo-controlled study in 116 subjects with MDD with at least one treatment failure found that a single dose of rapastinel 5 or 10 mg/kg IV reduced depressive symptoms as assessed by the Ham-D17 at days 1 through 7, with onset of action within 2 h, without psychotomimetic or other significant side effects (Preskorn et al. 2015). However, in three acute pivotal studies of rapastinel as an adjunctive treatment of MDD (RAP-MD-01,-02,-03), the rapastinel treatment arms did not differentiate from placebo on the primary and key secondary endpoints, though it was well tolerated, without psychotomimetic side effects (Allergan 2019). Furthermore, an interim analysis of the rapastinel relapse prevention study (RAP-MD-04) suggested the primary and key secondary endpoints were not met (Allergan 2019). Despite these negative clinical trials, it remains possible that rapastinel could prove effective as an alternative rapid agent with reduced side effects (Kato and Duman 2020).

11.5.3 Lanicemine

A low-trapping NMDA channel blocker, it was developed firstly as an iv adjunctive treatment for TRD with inconclusive results: in a 3-week, placebo-controlled phase IIB study of 152 randomized patients with moderate-to-severe MDD, repeated administration of lanicemine (100 or 150 mg per infusion) at 3-day intervals provided sustained antidepressant efficacy, without psychotomimetic effects (Sanacora et al. 2014), but a subsequent phase IIB study did not find significant differences in 302 randomized patients between lanicemine and placebo on any outcome measures related to MDD (Sanacora et al. 2017b). Lanicemine will be investigated as the active metabolite of BHV-5000 as oral treatment for TRD (Wilkinson and Sanacora 2019).

11.5.4 Other Compounds

Other mechanisms that have been or are being investigated as therapeutic targets for MD, without robust conclusive results so far, are (Wilkinson and Sanacora 2019): (1) a weaker antagonism of NMDAR (than ketamine) using dextromethorphan, in combination with an inhibitor of CYP2D6 to increase the bioavailability, namely quinidine or bupropion; (2) selective NR2B subunit NMDAR antagonism—Traxoprodil, EVT-101, Rislenemdaz; (3) different degrees of inhibition of NMDAR activity through glycine site partial agonism—Apimostinel, D-cycloserine; (4) selective antagonism at the glycine-binding site of the NMDAR NR1 subunit—AV-101; and (5) positive allosteric modulation of the AMPAR—tulrampator.

11.6 Conclusion and Future Perspectives

More than 60 years ago, the putative side effects of a new drug for tuberculosis (iproniazid), namely euphoria, psychoestimulation, increased appetite and sleep, were the rationale for a clinical study of that drug in patients with depression by Loomer, Saunders, and Kline in 1957 with good results (LOOMER et al. 1957), leading to the first (off-label) pharmacological treatment for depression, igniting the clinical research of a new class of treatments for depression (monoamine oxidase inhibitors and tricyclic antidepressants) and inspiring the monoamine hypothesis of depression (Hillhouse and Porter 2015).

Similarly, preliminary studies suggesting a possible antidepressant activity of a NMDAR antagonist was the rationale of the seminal work by Berman et al. in 2000 (Berman et al. 2000), as the first randomized, double-blinded clinical study specifically designed to determine the antidepressant effects of the ketamine, until then only used as an anesthetic. This has ignited intense efforts especially in the last 10 years for the clinical development of new glutamatergic and gabaergic modulators for the treatment of MDD, and basic and clinical research focused on the role of the glutamate and GABA systems in depression, with the result of the FDA approvals, in March 2019, of the first ketamine product (esketamine, nasal spray) for treatment-resistant depression and brexanolone as the first approved treatment for postpartum depression (FDA 2019a, b).

There are still many unresolved issues and limitations of the current evidence of the use of glutamatergic modulators for the treatment of MDD. Both the pharmacodynamics and the neurobiological underpinnings of the antidepressant effects of ketamine, esketamine, and other glutamatergic modulators remain largely unknown. Regarding pharmacokinetics, the glutamatergic modulators represent a new paradigm in comparison with the concept of traditional antidepressants administered daily to achieve almost constant blood levels: ketamine has a half-life of 2–4 h and apparently exerts neurobiological effects hours after, so the optimal pharmacokinetic

patterns and dosing schedule require further study (Wilkinson and Sanacora 2019). Also, more appropriate outcome measures to assess the efficacy of clinical trials with new glutamatergic rapid-acting antidepressants are required, given that current scales for depression—mainly HDRS and MADRS—are designed to capture change in symptoms over weeks or months and not hours or days (Wilkinson and Sanacora 2019). Worthy of note are the major safety concerns regarding the use of glutamatergic modulators as antidepressants (Molero et al. 2018): the potential for neurotoxicity associated with long-term use of ketamine, leading to possible neurocognitive impairment, also for nasal injuries or olfactory dysfunction associated with ketamine or esketamine intranasal route, and regarding potential physical side effects such as ulcerative cystitis and possible increased risk of bladder cancer. Further studies are needed to assess the tolerability profile of repeated doses of ketamine or esketamine in the long term, especially regarding the systemic and cardiovascular response and dissociative and psychotomimetic reactions. In addition, it is a priority to elucidate whether these treatments may induce substance abuse and how to control the risk of esketamine abuse specifically by means of new, appropriate galenical forms. Finally, the investigation of the efficacy of glutamatergic modulators in the treatment of severe depression with psychotic symptoms is an unmet need, given that psychotic symptoms are a common exclusion criterion in the proof-of-concept and phase III clinical trials with these molecules to date.

Future research may address these limitations to obtain a more precise knowledge of the antidepressant mechanisms of action, optimal dosing, posology and clinical indications of current and new glutamatergic modulators. The current evidence of the implication of the glutamatergic system in the pathophysiology of depression and the relevance of the gabaergic system to modulate the glutamatergic activity make it possible to anticipate the approval of new glutamatergic modulators for the treatment of major depression in the near future.

Financial Disclosures Without relevance to this work, PM is supported by Clinica Universidad de Navarra and has received research grants from the Ministry of Education (Spain), the Government of Navarra (Spain), the Spanish Foundation of Psychiatry and Mental Health and Astrazeneca. PM has been the principal investigator at Clinica Universidad de Navarra of several clinical trials of esketamine for depression supported by Janssen (one of them currently ongoing), is a clinical consultant for MedAvante-Prophase and has received speaker honoraria from or has been a consultant for AB-Biotics, Janssen, Novumed Roland Berger, and Scienta.

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Suggested Reading

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Chapter 12
Glutamate-Based Treatment for Amyotrophic Lateral Sclerosis/Motor Neuron Disease

A. Diana and P. Bongioanni

Abstract Glutamate is the most diffused amino acid in the brain throughout the human lifespan, since it is a unique actor in neuronal growth and differentiation, synaptic plasticity, learning and memory consolidation, arousal, and behavior. Upon certain circumstances, the glutamate homeostasis can be severely affected by the overproduction of this excitatory neurotransmitter, ultimately leading to neurodegenerative events via excitotoxic mechanisms. Therefore, the abnormal exposure to glutamate has been indicated the putative culprit in the onset and progression of motor neuron diseases (MNDs), a heterogeneous group of fatal neurodegenerative disorders encompassing the most common form called amyotrophic lateral sclerosis (ALS) also known as Lou Gehrig's disease. In ALS/MND neuronal excitotoxic demise is the final step of progressive muscle weakness and atrophy because of the upper or lower motor neuron dysregulation consistent with the degeneration of pyramidal neurons in the motor cortex, cranial motor neurons, and anterior horn cells in the spinal cord. This chapter aims at offering an overview of impaired molecules of the glutamatergic system in ALS/MND pathology. A relevant part of the topic is dedicated to discuss both the ongoing research with the available antiglutamatergic drugs and the alternative therapeutical strategies for feasible treatment of ALS/MND.

Keywords Neurodegenerative disease · Excitotoxicity · Glutamate receptors · Glutamate transporters · Glutamatergic signaling · Antiglutamatergic drugs

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_12

12.1 Introduction

Motor neuron diseases (MNDs) are a heterogeneous group of fatal neurodegenerative disorder whose causes are still largely unknown. MND leads to progressive muscle weakness and atrophy, with upper or lower motor neuron signs, or a mixture of them, due to degeneration of pyramidal neurons in the motor cortex, cranial motor neurons and anterior horn cells in the spinal cord. The amyotrophic lateral sclerosis (ALS) also known as Lou Gehrig's disease is the most common form. It involves both upper and lower motor neuron features. Less common variants involve a pure upper motor neuron presentation (primary lateral sclerosis) or a pure lower motor neuron presentation (progressive muscular atrophy).

Although the clinical outcome in ALS/MND has been identified with motor neuron degeneration, the disease is non-cell autonomous, meaning the involvement of astrocytes, microglia, and oligodendrocytes (Boillée et al. 2006; Ilieva et al. 2009; Haidet-Phillips et al. 2011).

ALS/MND has a mean incidence of two cases per 100,000 and prevalence of 5.4 per 100,000 individuals, although significant geographical differences have been reported (Logroscino et al. 2010; Chiò et al. 2013; Marin et al. 2017; Collaborators GBDMND 2018). Genetic discoveries have contributed to the advancement of pathophysiology understanding and broaden the original concept of ALS/MND, previously perceived in terms of neuromuscular disease into a more complex illness intermingled with cognitive decline leading to fronto-temporal dementia (FTD). As a matter of fact, primary symptoms of ALS/MND refer to motor dysregulation but soon other areas of the brain become prone to neurodegeneration. The overall process leads to mild to severe derangement of cognitive and behavioral functions in 40% to 60% of patients (Neary et al. 2000; Witgert et al. 2010; Ferrari et al. 2011; Phukan et al. 2012; Abrahams et al. 2014) and, more specifically, nearly 15% ALS/MND patients fulfill diagnostic criteria for FTD (Raaphorst et al. 2012). Despite 90% of ALS/MND cases are sporadic (sALS), the small remaining 10% fraction is inheritable (familial cases, fALS); hence, more than 30 genes (Chen et al. 2013; Zou et al. 2017; Mejzini et al. 2019) are directly implicated with the RNA processing, protein trafficking and degradation, cytoskeletal and axonal dynamics, mitochondrial metabolism and oxidation scavangers. Within those genes, superoxide dismutase 1 (SOD1), C9orf72, transactive response DNA-binding protein (TARDP) 43-kD, fused in sarcoma (FUS), are the four top more causative genes (Boylan 2015). Despite decades of intense clinical and basic research ALS/MND etiology remains elusive and, during this time, two hypotheses dealing with the site of disease onset have been advanced (Eisen et al. 1992; Kiernan et al. 2011). The first one is the dying-forward hypothesis, which accounts for an anterograde degeneration of motor neurons via glutamate excitotoxicity from the cortex. The second one is the dying-back hypothesis, which suggests that ALS/MND may start distally at the nerve terminal or the neuromuscular junction, continuing the progression toward the cell body due to the lack of specific neurotrophic factors. Indeed, it has also been

suggested that upper and lower motor neuron degeneration may be autonomously activated (Kiernan and Hudson 1991; Pamphlett et al. 1995).

Regardless of the anatomical point of initial injury, possible bias in the interneuronal connections has underpinned the dramatic role played by the modifications in excitatory glutamatergic neurotransmission for disease evolution concomitantly with enhanced neuronal vulnerability to excitotoxicity. In general terms, glutamate acts as an indirect neuronal killer via the sustained activation of glutamate receptors that, in turns, induces cationic influx, mitochondrial impairment, energy depletion, and oxidative stress leading to accumulation of reactive oxygen species (ROS) (Lipton 2008; Vincent and Mulle 2009; Connolly and Prehn 2015; Prentice et al. 2015).

12.2 Glutamatergic System and ALS/MND

Glutamate is the principal excitatory neurotransmitter of the central nervous system (CNS) and the most abundant amino acid in the brain. It is now widely accepted that it plays important roles in neuronal growth and differentiation, synaptic plasticity, learning and memory consolidation, arousal, and behavior. Glutamate overproduction not properly counterbalanced by buffering mechanisms has a striking relevance in neurological and psychiatric diseases encompassing depression, substance use disorder, schizophrenia, and other cognitive function and mood deficits (Zhou and Danbolt 2014). The critical threshold between glutamate physiology and overproduction is determined at the synaptic cleft due to the existing balance between glutamate clearance and its recycling through the glutamate-glutamine conversion. As already mentioned, it is transported inside vesicle compartments to the axonal terminals where, upon fusion to the presynaptic membrane, it can be released by exocytosis in synergy with anion channels and transporter reversal. The main actors of the glutamate metabolism are represented by glial cells, several types of receptors including excitatory amino acid transporters (EAATs 1-5), vesicular glutamate transporters (VGLUTs), alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, *N*-Methyl-D-aspartate (NMDA) receptors, glutamine-cystine exchangers (xCT), and various intracellular carriers. Any possible event that impairs their capacity to handle and control glutamate metabolism is reflected by a relevant modification of the excitatory transmission in the CNS that has been defined as excitotoxicity. This term has been coined (Olney and Sharpe 1969) to describe the neuronal cell death taking place by the abnormal exposure to glutamate or aspartate. This detrimental process is mediated by post-synaptic neurons hosting a plethora of ionotropic and metabotropic receptors that trigger intracellular cascade of signals. In contrast, glutamate transporters mainly distributed on the neighboring astrocytes by sequestering glutamate molecules are in charge of ending the molecular communication. In turn, the overstimulation of post-synaptic membrane gives rise to Ca^{2+} influx mediated by voltage-gated Ca^{2+} channels, NMDA, AMPA, and kainate (KA) receptors. Ca^{2+} entry will lead to dysregulation of mitochondria and endoplasmic reticulum (ER) functions, namely depolarization

of mitochondrial membrane, that precedes low-energy and oxidative state with excitotoxic ROS production (Van Den Bosch et al. 2006) ultimately leading to apoptotic demise (Lipton 2008; Caldeira et al. 2014). Despite the molecular intercellular mechanisms, when dealing with glutamatergic synapses, scientists have observed an unusual heterogeneity in the components of glutamatergic synapses that have a strong impact on the time courses of synaptic communication. In addition, the time course of glutamate dynamics can face some variations both because of the complexity of synaptic morphology including surrounding glial cells and the density of glutamate transporters (Jonas 2000). Therefore, the transition from physiological concentration and excitotoxicity favoring ALS/MND onset and progression is a breakpoint of the steady-state balance between glutamate secretion operated by neurons and glial cells (Martin 1992; Vesce et al. 1999; Montana et al. 2006). Finally and in order to understand the molecular components as potential targets for ALS/MND treatment, the glutamate neurotransmission can be recapitulated in the following steps: (1) Intracellular glutamate is stored into synaptic vesicles and released upon specific stimuli at the synaptic cleft determining a concentration peak; (2) This transient burst of glutamate is detected and transduced by glutamate receptors; (3) The excessive glutamate amount is removed from the synaptic space by the EAATs, mostly present in astrocytes, to prevent the overstimulation of the post-synaptic neurons. Remarkably, it should be highlighted that synaptic secretion of extracellular glutamate does not result in the major source of extracellular glutamate in the brain as shown in experiments where extracellular glutamate rate was not sensitive to the blockade of synaptic glutamate secretion (Timmerman and Westerink 1997; Jabaudon et al. 1999; Baker et al. 2002; Featherstone and Shippy 2008). This non-vesicular glutamate release has appeared mainly vehiculated by glial xCT cystine-glutamate antiport proteins in exchange with cystine import (La Bella et al. 2007).

12.3 Molecular Components of the Glutamate Neurotransmitter Signaling for ALS/MND

12.3.1 Glutamate Receptors

Glutamatergic neurotransmission is achieved by means of ionotropic (iGluRs) and metabotropic (mGluRs) receptors. The iGluRs are ligand-gated ion channels that drive the entry of sodium and potassium but massively calcium, while mGluRs are G protein-coupled receptors (GPCRs) that direct cellular homeostasis via G protein signaling cascades. Interestingly, glutamate does not show any preferential binding, suggesting that the two receptor families are synergically operating (Reiner and Levitz 2018). As a matter of fact, also mGluRs can induce Ca^{2+} unbalance since the rapid surge of cytosolic Ca^{2+} is able to evoke Ca^{2+}-induced Ca^{2+} release from intracellular stores of ER.

iGluRs belong to three distinct subfamilies such as NMDAR, AMPAR, and KAR (Nicoll et al. 1990). All of them share pre- and post-synaptic distribution and are made by the assembly of four subunits with different cation selectivity, Ca^{2+}-oriented only for NMDARs.

NMDAR is a heteromeric complex that interacts with multiple intracellular proteins by three different subunits: GluN1, GluN2, and GluN3. The functional NMDARs are heterotetramers composed of two GluN1 and typically two GluN2 subunits (Salussolia et al. 2011) that assemble to form an ion channel pore. However, the diversity in the subunit composition is responsible for changes in functional properties affecting both synaptic plasticity and stimuli triggered neuronal response (Paoletti 2011). Glutamate is only one of the agonists for NMDARs. Glycine and D-serine are co-agonists of the NMDARs, and their binding is a necessary requirement for the receptor activity. Since NMDARs have a relevant Ca^{2+} permeability and slow on/off kinetics (Cull-Candy et al. 2001), they definitely play a cardinal role in excitotoxicity. The Ca^{2+} cellular influx is significantly higher compared to the same cation entry in non-NMDA glutamate receptors and voltage-gated Ca^{2+} channels (Mody and MacDonald 1995; Petralia et al. 2010).

The possible impact of NMDAR involvement in the ALS/MND onset has been investigated by the pioneer work of Couriater and collaborators (Couratier et al. 1993) where cerebrospinal fluid (CSF) from ALS/MND patients was used for toxicity assessment (Sen et al. 2005). It resulted that AMPA/KA receptor antagonists were effective in blocking neurotoxicity, while NMDA antagonists were not protective. From that time, the decisive influence of NMDARs in ALS/MND has been progressively overlooked although the well recognized excitotoxicity due to receptor overstimulation.

In structural terms, AMPARs are organized in a tetrameric fashion by the combination of several subunit receptors named GluA1-GluA4, also known as GluR1-GluR4 and GluR-A to GluR-D (Wright and Vissel 2012). The Ca^{2+} entry of AMPARs is dependent on the presence of GluA2 subunit within the tetramer. Interestingly, it should be highlighted that astrocytes, in mutant SOD1, secrete molecules that downregulate the expression of GluA2 subunit in motor neurons causing excitotoxicity and cell death (Van Damme et al. 2005, 2007). A consistent set of data have been gained both in vivo and in vitro dealing with the effects of AMPARs in ALS/MND onset and progression. In SOD1-G93A mice, blocking AMPARs by specific antagonists has been useful to slow the progression of ALS/MND-like disease and meanwhile contributing to prolong the animal survival (Van Damme et al. 2003; Tortarolo et al. 2006), and even local infusion of Ca^{2+}-permeable AMPA channel blocker revealed positive effect for survival not only toward motor neurons but also astrocyte glutamate transporter (Yin et al. 2007). It has been also shown by iPS technology that motor neurons from patients carrying C9orf72 mutation were 100-fold more prone to glutamate toxicity (Donnelly et al. 2013). The definitive proof of the AMPAR dysregulation has been provided recently in post-mortem lower motor neurons located into the anterior horn of the spinal cord in ALS/MND. In particular, GluA1 and GluA2 receptor subunits exhibited a reverse expression: the former upregulated in sALS and mutant C9orf72 cases and the latter

downregulated in mutant SOD1 cases. At the higher CNS level, including prefrontal cortex, such heterogeneous and anomalous expression was widespread but strictly linked to the plasticity features of several brain areas by differential modulation of those two subunits (Gregory et al. 2020).

With regard to the last class of ionotropic receptors, KA receptors are tetrameric cation channels composed of five possible subunits with GluR5-7 necessary for functional channels and mixed to KA1 and KA2 (Kew and Kemp 2005). Similarly to AMPARs, upon glutamate exposure they allow ion flux resulting mostly impenetrable to Ca^{2+} ions. Although they find a preferential site in the membrane of postsynaptic neurons, they have been also recruited in the presynaptic domain (Chittajallu et al. 1996; Castillo et al. 1997).

12.3.2 Glutamate Transporters

Wealth of knowledge has been gathered in favor of a defective glutamate transport system in the onset and progression of ALS/MND that is intimately related to the astrocytic clearance function. As already mentioned, the dramatic consequences of the glutamate excess in the synaptic cleft make neurons highly vulnerable to excitotoxicity. For that purpose, astrocytes harbor high-affinity sodium-dependent glutamate transporters defined as EAATs in order to metabolize o recycle glutamate intersynaptic glutamate molecules. These transporters on the astrocytic membrane have been classified in EAAT1 or GLAST and EAAT2 or GLT-1 (Mahmoud et al. 2019). The sequestering capacity of astrocytes is extremely efficient, since the binding ratio has been calculated in terms of 10^{-7}/M/s (Takahashi et al. 2015). At the moment, multiple isoforms of EAAT2 (a, b, c) have been identified that variously change at the C-termini and, due to some slight variations at the 3'-UTR, they possibly face differential regulation (Lauriat and McInnes 2007). It is noteworthy that the complete knock-out of EAAT2 dramatically shortened life expectancy in a mouse model (Tanaka et al. 1997). The dramatic impact of astrocyte-mediated downregulation of EAAT2 has emerged in rodent models such as SOD1-G93A and TDP-43 possibly causing glutamate deposit in the extracellular space. In addition, focal loss of the EAAT2 glutamate transporter in the ventral horn of the spinal cord was concomitant to gliosis, but preceding motor neuron/axon degeneration. Such findings favor the hypothesis of a determinant role of this transporter in the apoptotic cellular cascade to cell death in ALS/MND (Howland et al. 2002; Tong et al. 2013).

Experimental studies have been performed to ascertain to what extent EAAT2 overexpression can counteract disease progression in genetically ALS/MND driven SOD1-G93A. Whilst it was effective on the disease onset, animals were neither protected from death (Guo et al. 2003) nor preserved from respiratory failure (Li et al. 2015). Biochemical assays provided further evidence for a link between the decrease in EAAT2 protein expression in homogenate fractions obtained from spinal cord in different murine SOD1 strains and neuronal demise (Bruijn et al.

1997; Bendotti et al. 2001). The dramatic decrease of this protein has been corroborated by the results obtained in ALS/MND patients where EAAT2 was downregulated both in the motor cortex and spinal cord by immunochemical detection (Rothstein et al. 1995). However, the same authors ended up with the conclusion that, at mRNA level, there was no quantitative change for EAAT1, EAAT2, or EAAT3 in ALS/MND motor cortex, even when patients presented both a massive loss of EAAT2 protein (95% decrease compared with control) and significative reduction of tissue glutamate transport (73% decrease compared with control) (Bristol and Rothstein 1996). Later on, Sasaki et al. (2000), in search for a relationship between EAAT1 and EAAT2 in ALS/MND patients, found the lack of immunoreactivity for EAAT2 correlated to the observed neuronal loss in the gray matter of the spinal cord while EAAT1 was still present. Interestingly, mild neuronal depletion was associated with a dense localization of EAAT2, whereas a strong reduction of the protein resulted when a consistent number of neurons disappeared. These data aimed at demonstrating a difference in EAAT1 and EAAT2 immunoreactivity in different stages of progression in ALS/MND, as a pathogenetic feature. The continued investigation of biochemical properties of EAAT2 molecule has brought to the discovery of three coding isoforms and multiple 5'- and 3'-UTRs with possible regulatory functions (Lauriat and McInnes 2007). Therefore, only recently it has been ascertained that the regulation of EAAT2a and EAAT2b transcripts is subjected to a different expression in transgenic SOD1-G93A rats, where EAAT2b was increased in young SOD1-G93A rats as compared to wild-type controls, but was prone to a slow decrease in both ventral and dorsal horns in the disease progression (Dumont et al. 2013). Remarkably and seemingly, despite it was detected a downregulation up to 95% of the overall EAAT2 levels in the motor cortex of ALS/MND patients, the specific EAAT2b isoform levels were raised by more than twofold (Maragakis et al. 2004).

12.4 Current Treatments and Modulators of Glutamatergic Signaling for ALS/MND

Due to the role of glutamate dysregulation in ALS/MND and its potential consequence of causing excitotoxicity, substantial research has been directed toward demonstrating the efficacy of antiglutamatergic drugs (Petrov et al. 2017; see Table 12.1).

12.4.1 Riluzole

Riluzole, a glutamate antagonist belonging to the benzothiazole class, acts as an anti-excitotoxic drug by inhibiting glutamate presynaptic release (thus promoting

Table 12.1 Antiglutamatergic drugs acting as modulators of glutamatergic signaling for ALS/MND

	Site of action	Mechanism of action
Riluzole	Presynaptic and postsynaptic neurons	Inactivation of fast voltage-dependent sodium channels
		Inhibition of voltage-gated calcium current
		Inactivation of slowing potassium channels
		Inhibition of protein kinase C
		Interfering with intracellular events that follow transmitter binding at excitatory amino acid receptors
Topiramate	Postsynaptic neurons	Blockade of voltage-dependent sodium and calcium channels
		Enhancement of GABA-dependent chloride inward currents
		Antagonism at glutamatergic AMPA and kainate receptors
Memantine	Postsynaptic neurons	Non-competitive antagonism at glutamatergic NMDA receptors
Talampanel	Postsynaptic neurons	Non-competitive antagonism at glutamatergic AMPA receptors
		Anti-inflammatory effects in the CNS
		Attenuation of caspase 3-mediated apoptosis
Ceftriaxone	Postsynaptic neurons	Increased glial transporter EAAT2 promoter activation and EAAT2 function in rodent brains

AMPA Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, *CNS* Central nervous system, *EAAT* Excitatory amino acid transporter, *GABA* Gamma-aminobutyric acid, *NMDA* *N*-Methyl-D-aspartate

glutamate re-uptake); inactivating fast voltage-dependent sodium channels (thus reducing hyperexcitability); inhibiting voltage-gated Ca^{2+} current; slowing potassium channel inactivation; inhibiting protein kinase C; and interfering with intracellular events that follow transmitter binding at excitatory amino acid receptors (Bellingham 2011).

Riluzole was approved for ALS/MND treatment, in the form of oral tablets, by the FDA in 1995, and subsequently licensed for use in other territories, including Europe (in 1996): the recommended dose is 50 mg twice daily, taken 1 h before or 2 h after a meal.

More recently, a novel, patented, oral liquid presentation of Riluzole, Teglutik® 5 mg/mL oral suspension, has been introduced (Dyer and Smith 2016): it has been reported to offer advantages in ALS/MND treatment, since dysphagia is present in about one-third of patients at onset, and over 80% develop this condition and/or need enteral feeding in later disease stages. The oral suspension potentially allows patients to continue Riluzole therapy for longer.

Human plasma Riluzole clinically effective concentrations are in the range between 0.5 and 2 μM: relatively high inter-individual variations have been reported (Groeneveld et al. 2003).

In a study of Riluzole plasma concentrations in ALS/MND patients, drug clearance was independent from dosage, treatment duration, age, and renal function, but was significantly lower in women compared to men, and in smokers compared to nonsmokers (Bruno et al. 1997), consistent with a report that mean plasma concentrations were higher in ALS/MND patients who were women or smokers (Groeneveld et al. 2003). Survival in a group of ALS/MND patients was found not to be correlated with individual Riluzole plasma levels (Groeneveld et al. 2007).

Four key publications related to randomized clinical trials (RCTs) conducted on Riluzole in ALS/MND patients were reported between 1994 and 2002 (Bensimon et al. 1994; Lacomblez et al. 1996; Yanagisawa et al. 1997; Bensimon et al. 2002): since then, no new RCTs appear to have been performed on Riluzole in ALS/MND patients.

Patients enrolled in the two trials which led to the licensing of Riluzole (Bensimon et al. 1994; Lacomblez et al. 1996) had been suffering from the disease for <5 years and had a baseline forced vital capacity (FVC) $\geq 60\%$.

In the first trial (Bensimon et al. 1994), 155 patients, recruited from France and Belgium, were followed up for at least 13 months after treatment with either 100 mg/day of Riluzole or placebo.

In the second trial (Lacomblez et al. 1996), 959 patients recruited from both North America and Europe were followed up for at least 12 and 18 months, respectively, after treatment with either 50, 100, or 200 mg/day of Riluzole or placebo: patients treated with 50 mg/day of active drug did not show a statistically significant difference compared with the placebo group, and the results of 200 mg/day were essentially identical to those with 100 mg/day. In the first trial (Bensimon et al. 1994), there was a 38,6% reduction in mortality, and the median survival time was 17.7 months in the active drug group vs 14.9 months in the placebo group; while in the second trial (Lacomblez et al. 1996), there was a 35% improvement in survival, and the median survival time was 16.5 months in the Riluzole group vs 13.5 months in the placebo group. There was no statistically significant difference in mortality at the end of the trials.

Data from the RCTs and subsequent meta-analyses, using death or tracheostomy as an endpoint, indicate that Riluzole at 100 mg/day had a modest beneficial effect on bulbar and limb function, but no effect on muscle strength, and typically extends survival by 2–3 months and increases the chance of an additional year of survival by 9% (Miller et al. 2012).

Riluzole treatment has been reported to have no effects on clinical EMG parameters commonly used to measure ALS/MND progression, including fasciculations, polyphasic motor unit potentials, increased motor fiber jitter and density, and increased amplitude and area of motor units (Desai et al. 1998). Patients often showed deficient paired pulse inhibition, presumably due to cortical hyperexcitability (Caramia et al. 2000): it has been reported that Riluzole treatment partially restored deficient paired pulse inhibition, but had no effect on paired pulse facilitation (Stefan et al. 2001), while other studies found that Riluzole treatment was without effect on motor evoked potentials or paired pulse inhibition (Caramia et al. 2000).

The above-mentioned survival data, however, do not take into consideration patients who survived longer than the 18- to 21-month follow-up period in the RCTs: over a decade on, there is substantial support for prolongation of survival times of patients following Riluzole therapy, based on retrospective and prospective studies utilizing clinical databases (Chiò et al. 2002; Miller et al. 2003; Traynor et al. 2003; Mitchell et al. 2006).

Meta-analyses on the RCTs have also been performed to examine the clinical and cost-effectiveness of Riluzole (Stewart et al. 2001): combined analysis revealed a hazard ratio of 0.89 (0.75–1.05), a small but positive benefit by Riluzole therapy.

A meta-analysis of RCTs concluded that drug safety was not a major concern in treatment, with nausea, asthenia, and elevated serum alanine transaminase being the only side effects which were significantly increased in patients receiving Riluzole, compared to those with placebo (Miller et al. 2012).

Asthenia is of particular interest, as clinically relevant Riluzole levels act to decrease motor neuron excitability and neurotransmitter release: Riluzole might contribute to asthenia by lowering individual motor neuron firing rate, therefore delaying fusion of muscle twitches in a single motor unit to tetany.

Caution in Riluzole administration has been recommended for patients with elevated serum transaminase levels or liver disease (Lacomblez et al. 2002).

Other adverse effects most commonly observed were somnolence, nervousness, circumoral paraesthesia, headache, itching, anorexia, diarrhea, depression (Debove et al. 2001; Groeneveld et al. 2003; Bensimon and Doble 2004). Incidence of such adverse drug effects was not related to Riluzole dosage in the range from 50 to 200 mg/day (Le Liboux et al. 1999; Groeneveld et al. 2003). Riluzole administration to elderly patients or advanced stage patients did not increase the incidence of adverse effects (Le Liboux et al. 1999; Bensimon et al. 2002).

Subsequent to the RCTs, more than ten independent retrospective and prospective studies on ALS/MND patients utilizing clinical databases from patients in the "real-world" have been carried out. Riluzole administration in ALS/MND patients was well tolerated for periods of up to 7 years (Lacomblez et al. 2002). Open label, nonrandomized studies have suggested that Riluzole treatment at earlier disease stages may have greater benefits (Riviere et al. 1998; Traynor et al. 2003; Zoing et al. 2006). Patients with bulbar onset may also benefit more from drug treatment (Traynor et al. 2003; Zoccolella et al. 2007), and lifespan increase may be greater in patients with symptom onset at older ages (Zoccolella et al. 2007).

Individual studies suggest that Riluzole therapy produced prolongation of the time spent in the initial or milder disease stages, but had little effect on time spent in advanced or severe disease stages (Riviere et al. 1998).

On the other hand, a recent data analysis from the original Riluzole dose-ranging RCT (Lacomblez et al. 1996) on 959 patients, by using the King's clinical staging system (Roche et al. 2012), reported that Riluzole prolonged survival in the ALS Stage 4 (corresponding to either nutritional failure with 10% of premorbid weight loss due to dysphagia, sufficient to require gastrostomy, or substantial respiratory failure fulfilling guidelines for needing non-invasive ventilation), and that most of the benefits occurred during this stage. Moreover, there was no difference in time

from trial Stages 2 or 3 to the next stages or death between the active drug and the placebo treatment groups (Fang et al. 2018).

Such findings were robust to the method of analysis and independent of the stage at which treatment was started: they imply that Riluzole survival benefits were achieved by extending Stage 4, not by prolonging Stages 2 or 3, or generally slowing disease. Furthermore, the "selective" Stage 4 extension would help to explain the original report of improvement in survival without a concomitant effect on function (Lacomblez et al. 1996), since function at this stage is limited, and a flattening of the slope of functional decline would be hard to detect.

The use of the "real world" evidence is crucial to ensure that the results obtained in RCTs translate into tangible benefits in the patients' population; based largely on retrospective/prospective analysis of huge clinical databases (encompassing more than 5000 patients), it suggests significant enhancement of median survival by up to 19 months in Riluzole-treated ALS/MND patients with the most remarkable effects in patients who started the drug early in the disease: such an information should assist prescribers, patients, and caregivers in effectively managing ALS/MND treatment. Not only, but such data are more impressive, by considering that the clinical databases comprised patient populations ranging from the United Kingdom, Ireland and Italy to the USA, Taiwan, and China: the fact that differences (due to likely differences in healthcare systems and ancillary care, such as nutritional supplementation) were not found supports the relevance of such data (Meininger 2002).

Besides Riluzole other drugs which have targeted glutamatergic transmission as their primary mechanism of action were used over time in ALS/MND treatment.

12.4.2 Topiramate

Topiramate, a well-known antiepileptic drug, is effective against focal onset seizures and generalized onset tonic-clonic seizures and as a prophylactic treatment of migraine (Spritzer et al. 2016). Topiramate has several mechanisms of action: although none of them has been pointed out as the principal one, blockade of voltage-dependent sodium and calcium channels, enhancement of GABA-dependent chloride inward currents, and antagonism at glutamatergic AMPA and kainate receptors have received most attention (Shank et al. 2000). Moreover, some studies have shown that it has neuroprotective properties (Kudin et al. 2004).

A double-blind, placebo-controlled, multicenter clinical trial (Cudkowicz et al. 2003) was carried out in 296 ALS patients, randomized (2:1) to receive Topiramate (maximum tolerated dose up to 800 mg/day) or placebo for 12 months.

Patients treated with the active drug showed a significantly ($p = 0.012$) faster decrease in arm strength (33.3%) as measured by the maximum voluntary isometric contraction, but no significant differences—as compared to placebo group—in the decline of FVC and ALS functional rating scale (ALSFRS) or in survival.

Topiramate was associated with an increased frequency of anorexia, depression, diarrhea, ecchymosis, nausea, kidney calculus, paresthesia, taste perversion,

thinking abnormalities, weight loss, and abnormal blood clotting (pulmonary embolism and deep venous thrombosis).

Since Topiramate, at the dose studied, did not have a beneficial effect for ALS/MND patients, further studies on such a drug (at least at a daily dose of 800 mg) were not warranted (Cudkowicz et al. 2003).

12.4.3 Memantine

Memantine, a moderate-affinity voltage-dependent non-competitive antagonist of glutamatergic NMDA receptors, has been shown to protect neurons against glutamate-induced in vitro toxicity (Chen and Lipton 1997). It inhibits and reverses the abnormal tau hyperphosphorylation leading to protein aggregation and the disassembly of microtubules (Li et al. 2004). Memantine, currently approved for the treatment of Alzheimer's Disease, has been shown to prolong survival in a SOD1-G93A mouse model for ALS/MND (Wang and Zhang 2005).

A phase II/III, 12-month, double-blinded, single center, randomized, parallel, placebo-control clinical trial with 63 patients also treated with Riluzole (50 mg twice a day) failed to demonstrate any effect of 20-mg daily memantine on both primary and secondary endpoints in clinically probable, probable-laboratory supported or definite ALS patients of less than 36-month disease duration and progression over a 1-month lead-in period (De Carvalho et al. 2010). Primary endpoint was 12-month ALSFRS decline; secondary endpoints were FVC, manual muscle testing, visual analogue scale, quality of life, motor unit number estimation, and neurophysiological index (De Carvalho et al. 2010). Memantine did not show more adverse events or lab (blood chemistry) changes than placebo. Therefore, the study showed that memantine was well tolerated and safe, but with no evidence of efficacy, although the authors could not exclude a positive outcome on survival.

In a pilot trial (Levine et al. 2010) in 20 patients suffering from sporadic clinically probable or definite ALS, receiving Riluzole (50 mg daily) and Memantine (10 mg twice a day) for 18 months, changes in CSF biomarkers during drug therapy were studied. Memantine was well tolerated and there was a strong correlation between CSF tau levels and disease progression. Since such a study had no a placebo arm, it is difficult to draw conclusions about the significance of CSF tau decline during memantine treatment: further studies would be needed to assay levels of CSF tau over time within individual ALS/MND patients. Moreover, no conclusions about the efficacy of memantine in slowing down the disease course can be drawn, given the open label nature of the trial.

12.4.4 Talampanel

Talampanel, a small molecule with good blood-brain barrier (BBB) penetration, is an orally active non-competitive antagonist of AMPA receptors mediating glutamate-induced excitotoxicity to motor neurons (Van Den Bosch et al. 2000). It is a member of the family of the benzodiazepines; it has independent anti-inflammatory effects (Greene et al. 2008) in the CNS and attenuates caspase 3-mediated apoptosis (Denes et al. 2006).

Talampanel has been tested in human trials of epilepsy and was found to have significant anticonvulsant properties, demonstrating bioavailability within the CNS (Langan et al. 2003).

A phase II, double-blind, placebo-controlled, multicenter, randomized clinical trial of 9-month treatment duration, performed on 59 definite or probable ALS patients (40 subjects receiving 50-mg orally thrice a day active drug and 19 subjects placebo), showed a trend toward slower decline in ALSFRS and isometric muscle strength in Talampanel-treated patients (Pascuzzi et al. 2010). Talampanel was well tolerated: mortality rates and drug discontinuation rates were similar in both groups of active drug- or placebo-treated patients; although some adverse events occurred more frequently in the Talampanel group, the rate of subject drop-out after 9 months did not exceed that seen in other trials (Pascuzzi et al. 2010).

The data for the failed Phase 3 study, recruiting 559 patients (trial ID# NCT00696332) was not publicly disclosed.

In addition, a patent disclosed evidence of the efficacy, tolerability, and safety of 150-mg daily doses of drug in patients afflicted with ALS/MND (Ben-Ami et al. 2009): Talampanel had beneficial effects on the rate of functional decline and the progression of symptoms: neuroprotective effects of Talampanel were only present when it was applied during the early disease stage.

12.4.5 Ceftriaxone

Ceftriaxone is an FDA-approved beta-lactam antibiotic with good CNS penetration through the BBB.

It increases glial transporter EAAT2 promoter activation and EAAT2 activity in rodent brains (Rothstein et al. 2005); protects motor neurons in culture from excitotoxicity (Vincent et al. 2005); and has antioxidant activity (Tikka et al. 2001). Ceftriaxone administered to SOD1-G93A mice at disease onset slowed the disease course, preserved strength, delayed weight loss, and prolonged survival (10 days longer); moreover, SOD1-G93A mice treated before disease onset had a decreased motor neuron loss after 2 weeks (Rothstein et al. 2005).

In addition to the preclinical data supporting its beneficial effects in ALS/MND models, Ceftriaxone has been reported as neuroprotective in vitro and in vivo in other neurologic diseases, including spinal muscular atrophy (Nizzardo et al. 2011),

Huntington's Disease (Miller et al. 2008), ischemic stroke (Thone-Reineke et al. 2008), and multiple sclerosis (Melzer et al. 2008).

Ceftriaxone is the only intravenous beta-lactam antibiotic which was investigated in a large-scale non-stop Phases I-II randomized clinical trial recruiting 513 ALS patients (Berry et al. 2013; Cudkowicz et al. 2014), using a novel 3-stage design: Stage 1 determined the CSF pharmacokinetics; Stage 2 evaluated safety and tolerability for 20 weeks; based on the data of the two previous stages, drug dosage was established for Stage 3 efficacy testing.

Stage 1 analysis showed linear pharmacokinetics and demonstrated that both Ceftriaxone dosage levels (2 g and 4 g daily) achieved CSF concentrations of $\geq$1 μM (0.55 μg/mL), namely those of in vitro efficacy: drug holidays of up to 3 days were possible while maintaining effective CSF drug concentrations ($\geq$ 1 μM) in patients receiving high doses (4 g/die).

Tolerability findings pointed out that Ceftriaxone at daily dosages up to 4 g was well tolerated for 20 weeks. Biliary adverse events (biliary sludge/cholelithiasis) were more common with the active drug, but they were not dose-dependent and improved with biliary acid therapy; other adverse events did not occur more commonly with Ceftriaxone treatment as compared to placebo.

Nevertheless, the study failed to demonstrate significant improvement in survival and functional outcomes in ALS/MND patients (Berry et al. 2013; Cudkowicz et al. 2014).

Functional decline was reported slower in patients belonging to the high dose (4 mg daily) group as compared to those with placebo ($p = 0.0416$): unfortunately, such a decrease in functional decline was not replicated in the Stage 3 interim or final analysis, nor differences in survival were observed. Furthermore, adverse event rates were significantly higher in the active drug group for gastrointestinal, hepatobiliary, and blood or bone marrow involvement: patients receiving Ceftriaxone also had more hepatobiliary serious adverse events, but fewer infection-related serious adverse events.

Since Ceftriaxone was ineffective in treating ALS/MND patients, it remains to be clarified whether EAAT2 upregulation is no longer a useful clinical target. The actual efficacy of drug in increasing EAAT2 in the CNS was not determined as no markers existed at the time of the study. On the other hand, animal models also demonstrated Ceftriaxone efficacy only when administered before symptom onset. Perhaps it would be more valuable for pre-onset fALS carriers: stratification by mutation status, onset type, or cognition status might also yield interesting results (Van Den Berg 2014).

An alternative explanation for the failure of Ceftriaxone in clinical trials might be the existence of a secondary EAAT2-mediated neurotoxicity pathway: EAAT2 is cleaved by caspase 3 in ALS/MND, leading to the generation of a C-terminal fragment/end (CTE), which was found to be sumoylated (Gibb et al. 2007). Transfection of an artificially fused CTE-SUMO1 construct induces astrocytes to secrete toxic factors for motor neurons (Foran et al. 2011). Theoretically, such a pathway would be upregulated with many of the EAAT2-targeted treatments, potentially

preventing their success by coupling induction of decreased excitotoxicity with an increased secretion of toxic factors.

12.5 Future Developments and Modulators of Glutamatergic Signaling for ALS/MND

The fundamental issues and challenges of developing new effective drugs for treatment of ALS/MND patients are related to inadequate animal models and preclinical experimental designs; pharmacokinetic differences between rodents and humans; the genetic complexity of ALS/MND disease and genotypic feature discrepancies in recognizing the disease subtypes; the heterogeneity among patients and diagnostic delays; poorly sensitive biomarkers; RCTs faulty designs, together with the lack of distinction between null versus negative effects, and the lack of focus on clinical significance instead of just statistical significance.

Measures and strategies, to reduce the number of false positives in preclinical studies and thereby prevent unwarranted clinical trials, and improve RCTs in ALS/MND patients, can be suggested: (1) developing novel animal models (Perrin 2014) to better understand disease pathophysiology, according to guidelines for preclinical studies (Kilkenny et al. 2010); (2) using correct experimental designs, statistical models, and measures; (3) determining drug accessibility to target tissue/region, right time of treatment, better dose and dose response-curve; (4) differentiating between null and negative drug effects; (5) adapting innovative study designs and careful patients' enrollment to cut cost and increase RCT robustness (Mitsumoto et al. 2014); (6) classifying phenotypic variations among patients and stratificating patients in different groups for RCTs; (7) evaluating multiple doses over different time-spans to point out the correct dose (Gordon and Meininger 2011); (8) incorporating mathematical modeling and computational biology to discover gene–gene and gene–environment interaction; (9) developing new models for drug screenings (Jaiswal 2017); (10) testing multidrug therapy and novel treatment approaches, such as those with stem cells, antisense oligonucleotides, or mitochondrial replacement.

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Chapter 13
Modulation of Glutamate for Chronic Pain Management

Kathleen F. Holton

Abstract Glutamatergic neurotransmission is strongly implicated in both normal pain neurotransmission and the transition to chronic pain and central sensitization. As such, there is great interest in identifying optimal treatment options which beneficially reduce pain with limited side effects. Potential options include antagonism of ionotropic and Group I metabotropic glutamate (mGlu) receptors, agonism of Group II and III mGlu receptors, modulation of transporter function, reduction in neuroimmune cytokines which affect glutamate, improvement in glutamatergic and GABA neurotransmission through dietary modulation, and other non-pharmacological approaches such as electroacupuncture and exercise. Current pharmacological treatment options are limited due to widespread distribution of glutamate action in the body, making side effects very common. Non-pharmacological treatment options such as dietary intervention may be good adjunct treatments due to the positive effects on glutamatergic neurotransmission and reduction of inflammation, with little to no side effects. Current pharmacological and non-pharmacological treatment options which affect glutamatergic neurotransmission for the treatment of chronic pain are reviewed.

Keywords Glutamate · Chronic pain · Central sensitization · Treatment · Ionotropic glutamate receptor · Metabotropic glutamate receptors · Diet

13.1 Introduction

Pain is considered an essential protective mechanism in the body when happening acutely. However, chronic pain, related to nerve injury or inflammation, can lead to reduced quality of life and disability, and is now considered a major public health problem (Blyth et al. 2015). The prevalence of chronic pain has been estimated to

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_13

fall between 30 and 50% of the worldwide population (Inoue et al. 2015; Fayaz et al. 2016; de Souza et al. 2017), with higher prevalence rates in women and the elderly (de Souza et al. 2017).

Patients suffering with chronic pain will typically report this sensation after both painful stimuli (hyperalgesia) and non-painful stimuli (allodynia), manifesting in a greater pain response than a normal healthy individual (Sandkuhler 2009). This phenomenon has been associated with central sensitization, where the central nervous system undergoes neuroplasticity to support a persistent state of high reactivity, which accumulating evidence suggests may also be associated with neuroinflammation (Ji et al. 2018). Widespread chronic pain commonly presents with other comorbid symptoms including headache/migraine, cognitive dysfunction, sleep problems, mood dysregulation, chronic fatigue, and gastrointestinal issues, which are thought to be related to central sensitization (Yunus 2007). These comorbid symptoms can make chronic pain even more debilitating. One study characterized high-impact chronic pain in the USA and found that the likelihood of disability from chronic pain was over 4 times higher than from other chronic conditions (OR (95% CI) = 4.43 (3.73–5.26) (Pitcher et al. 2019).

Opioids have been used for decades as an effective short-term treatment for acute pain, and this prior success led to several prominent pain specialists in the 1980s to suggest that opioids also be prescribed for the treatment of chronic pain (Meldrum 2016). It is now well known that this increased prescribing practice has contributed to the opioid epidemic now being faced in the USA (Floyd and Warren 2018). The opioid epidemic has brought to light the imperative need for alternative ways to treat chronic pain. Glutamate, the most ubiquitous neurotransmitter in mammalian systems, is well-known for its contribution to pain signaling. This knowledge has led to a growing interest in identifying ways to modulate glutamatergic neurotransmission for the treatment of chronic pain.

13.2 The Glutamatergic System in Chronic Pain

Glutamate functions as a neurotransmitter throughout the body, with receptors found not only in the CNS, but also in the immune system, pancreas, heart, kidney, lungs, gastrointestinal tract, and skin (Gill and Pulido 2001). The wide-ranging location of these receptors may be the reason for the diverse symptom presentation in widespread chronic pain conditions, as well as the varying side effect profiles of medications affecting this system.

Glutamate plays a major role in pain neurotransmission, with receptors found in the sensory part of the periphery, the spinal column (Aδ and C fibers), and in the pain processing areas of the brain, such as the anterior cingulate cortex (ACC), insular cortex (IC), primary somatosensory cortex (S1), secondary somatosensory cortex (S2), and prefrontal cortex (PFC) (Zhuo 2017). Additionally, the amygdala, a brain region responsible for the pain-related emotional responses and anxiety-like behavior, also uses glutamate in its functioning (Neugebauer 2015).

Peripheral pain signaling can activate Aδ and C fibers in the spinal cord, releasing glutamate in the spinal dorsal horn, along with pain-related neuropeptides like substance P and neurokinin A. These neuropeptides and glutamate then activate spinal dorsal horn neurons, triggering afferent signaling to the thalamus which then signals to the brain areas mentioned above (Li et al. 2019). It is important to note that substance P has the ability to create permeability in the blood-brain barrier (BBB) (Vink et al. 2017), in addition to the BBB being susceptible to neuroinflammatory effects from mast cells and glia cells (Skaper 2016). Thus, prolonged pain signaling and neuroinflammation can lead to increased susceptibility to toxins circulating in the bloodstream.

There are two major types of glutamate receptors, ionotropic and metabotropic. The ionotropic receptors include NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazopropionic acid), and kainate receptors ((Zhuo 2017). For an in-depth review of the contribution of cortical ionotropic glutamate receptors in chronic pain, the reader is encouraged to see Zhuo (2017). Additionally, recent research has also shown that metabotropic receptors have the ability to affect pain through their ability to modulate the action of the ionotropic receptors (Palazzo et al. 2014; Cavallone et al. 2020).

The NMDA receptor has the most complex modulation of the ionotropic receptors. As opposed to simple transport of sodium and potassium which is used for AMPA and kainate function, the NMDA receptor has more complex regulation and uses calcium signaling. For NMDA activation, glutamate must be present along with glycine (Curras and Pallotta 1996). Magnesium can block the calcium channel of the NMDA receptor, serving as a soft inhibitor to activation (Kirkland et al. 2018) and this block can be removed by sufficient membrane depolarization (Latremoliere and Woolf 2009). Additionally, zinc is co-released with glutamate at the synapse, and also has the ability to modulate function of the NMDA receptor (Mony et al. 2009). Phencyclidine (PCP) and ketamine are both well-known strong antagonists of the NMDA receptor and have been used as drugs of abuse (Cadinu et al. 2018). Thus, this receptor can be modulated by both pharmacological and nutritional means.

While all three ionotropic receptors have some impact on chronic pain, the NMDA receptor has been most highly implicated in pain, being especially important for long-term potentiation (LTP), which is a strengthening of synapses based on patterns of signals, which results in long-term strengthening of signal transmission between neurons. While LTP is vital for memory and learning processes in the hippocampus, it can have negative consequences for chronic pain when it occurs in the spinal cord, ACC, and IC (Li et al. 2019), leading to central sensitization (Latremoliere and Woolf 2009). Glial activation has also been implicated in the pathogenesis of chronic pain, with some authors having suggested that chronic pain may manifest as a "gliopathy" or dysfunction of glial cells, which has been connected to neuroinflammation (Ji et al. 2013). Gliopathy is characterized by downregulation of astrocytic glutamate transporters in spinal astrocytes, resulting in glutamate accumulation and excessive excitation of neurons (Sung et al. 2003).

Historically, it was believed that central sensitization resulted from sustained persistent nociceptive input from the periphery; however, it is now understood that central sensitization can also occur from changes in the properties of neurons in the central nervous system (Latremoliere and Woolf 2009). For example, traumatic brain injury and multiple sclerosis can cause central sensitization, neuroinflammation, and chronic pain, without any peripheral trauma or damage (Hains and Waxman 2006).

13.3 Molecular Targets of the Glutamate Neurotransmitter System

In order to identify optimal molecular glutamatergic targets for the treatment of chronic pain, it is imperative to understand potential areas of modulation. Drug development has been somewhat inhibited due to the wide distribution of glutamate receptors throughout the peripheral and central nervous systems, making it difficult to treat chronic pain via modulation of glutamate, without causing side effects in some bodily functions.

Potential drug targets include antagonists of the ionotropic and Group I mGluRs, agonists of Group II and III mGluRs, modulation of NMDA receptor function, inhibition of calcium or sodium channels of glutamate receptors, upregulation of glutamate transporters which help clear glutamate from the synaptic cleft to prevent excitotoxicity, upregulation of the inhibitory neurotransmitter GABA to off-set the excitatory effects of glutamate, or modulation of neuroimmune cytokines to reduce

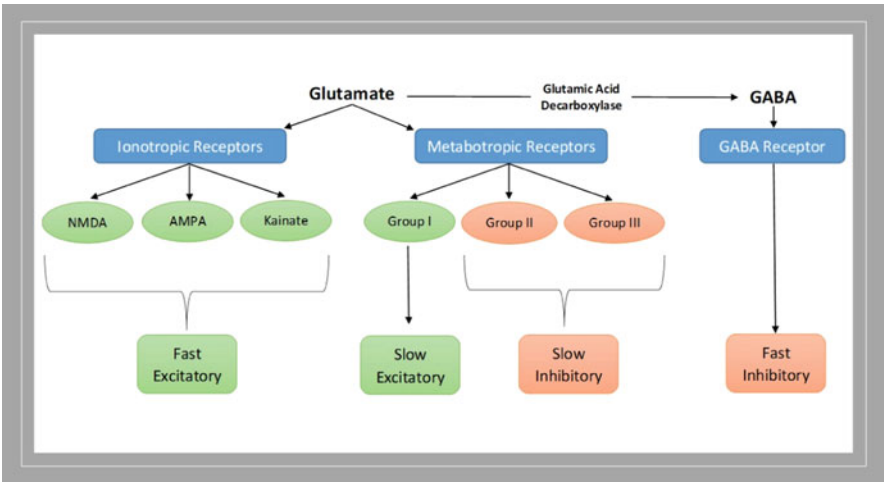

Fig. 13.1 Glutamate and GABA receptors

neuroinflammation and the downstream effects on excitatory neurotransmission. Figure 13.1 shows the differential effects of glutamate and GABA receptors.

13.4 Emerging Treatments Related to Glutamate Modulation

13.4.1 Pharmacological Effects on Glutamate Neurotransmission

There are five types of pharmacological approaches currently being used to treat chronic pain. Each of these is discussed below, including specific examples of medications, the types of pain they are being used to treat, and side effects or other limitations to their use.

13.4.2 NMDA Antagonists

NMDA antagonists have demonstrated varying benefit for the treatment of chronic pain (Nicol et al. 2017). As discussed above, due to the location of glutamate receptors throughout the body, antagonism of ionotropic receptors, such as the NMDA receptor, can cause widespread side effects in the body, in addition to having the potential for abuse (Liu et al. 2016). However, due to how strongly the NMDA receptor is implicated in chronic pain and central sensitization, there is intense interest in modulation of this receptor.

Ketamine has been tested in many types of neuropathic pain, with demonstrated benefit in 12 of 13 intravenous studies and 2 of 3 studies using oral ketamine, but only in 1 of 5 studies using the topical version (Aiyer et al. 2018). Ketamine is the only medication which has demonstrated effectiveness in treating chronic regional pain syndrome (CRPS) (Sigtermans et al. 2009; Schwartzman et al. 2009) and has also demonstrated positive intra-group change effects in fibromyalgia (Olivan-Blazquez et al. 2014). Use of IV ketamine for pain is considered an off-label use, and as such, is not covered by insurance, and is also expensive to set up, which limits the feasibility and accessibility of this medication (Aiyer et al. 2018). The downsides of this approach include the necessity for tight control of drug delivery, mainly by IV administration, and its high potential for abuse (Liu et al. 2016); however, as demonstrated above, IV ketamine has the most studies supporting its use. Side effects of ketamine, like other NMDA antagonists, affect multiple body regions and include psychedelic effects, nausea/vomiting, somnolence, heart effects, bladder/renal complications and infrequent hepatotoxicity (Niesters et al. 2013). Ketamine sensitization (necessary for addiction to occur) has been shown to be both robust and reliable in animal studies (Trujillo and Heller 2020). Nevertheless, in

2018, consensus guidelines were published by the American Society of Regional Anesthesia and Pain Medicine, the American Academy of Pain Medicine, and the American Society of Anesthesiologists, supporting the intravenous use of ketamine for the treatment of chronic pain. The group observed that the level of evidence varied by condition and dose range, with higher doses and more frequent infusions being associated with the greatest risk of side effects (Cohen et al. 2018).

Dextromethorphan is a medication most often seen as an ingredient in cough suppressant over-the-counter medication. It has also been tested as a treatment for chronic pain, and was found to be effective in reducing pain intensity in diabetic neuropathy (Sang et al. 2002; Nelson et al. 1997). Dextromethorphan is thought to be a safer alternative to ketamine, but can cause psychological addiction, and is limited by side effects including skin issues, gastrointestinal symptoms, vestibular effects, confusion, sedation, nervousness, and hallucinations (Ziaee et al. 2005).

Carbamazepine is a medication typically used to treat seizure disorders, but which is also used for treatment of neuropathic pain. Most of the studies have been conducted on trigeminal neuralgia, with consistent benefit being demonstrated in most studies, as well as some suggested benefit in diabetic neuropathy (Aiyer et al. 2018). Carbamazepine appears to have a narrow therapeutic index and substantial interethnic and interindividual variability in effectiveness. Some serious adverse effects of this medication have been reported, and include decreased bone marrow function, suicidality, and potentially life-threatening hypersensitivity reactions, such as Stevens-Johnson syndrome and toxic epidermal necrolysis (Jaramillo et al. 2014).

Interestingly, methadone, which is typically known for its action as a synthetic opioid agonist, has been shown to also be a potent NMDA antagonist (Davis and Inturrisi 1999; Shimoyama et al. 1997). Three out of six studies have demonstrated efficacy of methadone for the treatment of neuropathic pain and chronic cancer pain (Aiyer et al. 2018), but use of this medication is limited by risk of cardiac complications (Faisal and Jacques 2017) and the stigmas surrounding its use, which have been tied to discrimination, stereotypes and prejudice (Earnshaw et al. 2013).

Lastly, valproic acid is an anticonvulsant which has also been shown to block excitatory responses via NMDARs. It has good evidence for neuropathic pain relief, with 4 of 5 studies demonstrating benefit (Aiyer et al. 2018). Valproic acid is also being used for migraine prevention (Shelton and Connelly 1996) and is used as an intravenous treatment for acute migraine treatment (Waberzinek et al. 2007). However, this medication is also limited by its side effect profile which includes dermatological effects on skin, alopecia, organ toxicity, weight gain, and worsening of triglyceride, glucose, and cholesterol measures (Dreifuss and Langer 1988).

Overall, a systematic review of these NMDA antagonists reported that IV ketamine and oral carbamazepine currently have the most evidence of efficacy for neuropathic pain conditions (Aiyer et al. 2018). Even though there are substantial side effects to antagonism of the NMDA receptor, the benefits demonstrated so far are great enough to elicit continued research in this area.

13.4.3 Modulation of Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) are gaining attention as a potential candidate for drug development for chronic pain (Cavallone et al. 2020; Palazzo et al. 2014). These mGluRs are able to modulate presynaptic neurotransmission by influencing ionotropic neurotransmission. These are classified into three groups based on their pharmacological profile and effects on signal transduction (Crupi et al. 2019). Group I mGluRs (including mGluR1 and mGluR5) normally increase excitation, whereas Group II mGluRs (including mGluR2 and mGluR3) and Group III mGluRs (including mGluR4, mGluR6, mGluR7, mGluR8) normally inhibit ionotropic glutamatergic neurotransmission (Conn and Pin 1997). Thus, work is focusing on antagonists to specific Group I receptors and agonists to specific mGluRs in Group II and III. For example, Cavallone and colleagues recently reported on research testing the mGlu5 antagonist Fenobam in humans, since this compound has shown analgesic effects in a number of mouse pain models (Montana et al. 2011; Crock et al. 2012). Unfortunately, they did not observe any lasting antihyperalgesic or antinociceptive effects of fenobam compared to placebo in a skin sensitization model in humans; and the authors recommend further clinical trials with other compounds with better pharmacokinetic profiles (Cavallone et al. 2020). Animal models have also demonstrated promising analgesic effects from agonists of Group II and III receptors, such as (*S*)-3,4-DCPG (Palazzo et al. 2014), but, to the best of our knowledge, no human studies using this compound have yet been conducted. Thus, development of drugs affecting mGluRs is still in its infancy and more work is needed to help improve the selectivity of these compounds to help bypass the differential pain effects in animals versus humans.

13.4.4 Modulation of Glutamate Transporters

There is great interest in ways to modulate excitatory amino acid transporters as a treatment for chronic pain. These transporters clear glutamate from the synaptic cleft, helping to prevent excitotoxicity. The glutamate transporters include EAAT1/GLAST, EAAT2/GLT-1, and EAAT3-EAAT5, with the first two facilitating >95% of glutamate transport in astrocytes, oligodendrocytes, and microglia (Danbolt 2001; Domercq and Matute 1999; Xin et al. 2009). Abnormal function of these transporters (such as due to energy deprivation via caloric restriction) is known to lead to excitotoxic neuronal damage (Kanai et al. 2013). One exciting line of research is examining the effects of beta-lactam antibiotics on glutamate transport function. Ceftriaxone is a broad-spectrum beta-lactam antibiotic that enhances glutamate reuptake through increased expression of excitatory amino acid transporter 2 (GLT1/EAAT2) (Lee et al. 2008). Ceftriaxone has also been shown to induce expression of the cystine/glutamate exchanger, which increases cysteine availability for the production of the potent antioxidant glutathione, leading to additional

antioxidant neuroprotective effects (Gegelashvili and Bjerrum 2014). However, long-term antibiotic treatment is not ideal for chronic pain, so work on alternative beta-lactam compounds which are devoid of antibiotic properties is in development. For example, clavulanic acid is a beta-lactam compound under investigation for the treatment of pain, which has been shown to upregulate glutamate transporters in rat studies (Kristensen et al. 2018). This is a very promising area of drug development.

In addition to beta-lactam antibiotic type compounds, other drugs which upregulate glutamate transport include riluzole and amitriptyline. Riluzole is a medication approved for use in ALS which also enhances the reuptake of glutamate by inducing GLT1 expression (Carbone et al. 2012). Unfortunately, riluzole is a relatively toxic compound, and as such, is again limited mostly to its use in serious neurodegenerative conditions (Clark and Vissel 2016). In contrast, amitriptyline has been used for years as an effective treatment for chronic pain, with lower doses being needed for pain than for treating depression (Moore et al. 2012). The exact mechanism of the antinociceptive effects of amitriptyline is unknown, but some studies have suggested that it has the ability to modulate the functional expression of glutamate transporters in the spinal cord (Mao and Yang 2010). Amitriptyline appears to be able to cause fast translocation of three major transporters, which could be mediated through activation of the transcription factor Nf-κB (Tai et al. 2008). Thus, modulation of glutamate transporters is already being used as an effective treatment for chronic pain and also has some promising new drug candidates like clavulanic acid in the pipeline.

13.4.5 Inhibition of Calcium & Sodium Channels

The gabapentinoids are a class of antiepileptic medications, including pregabalin and gabapentin, which were originally developed as GABA analogues. However, the medications ended up having no direct effects on GABA receptors, and instead appear to reduce excitatory synaptic transmission through inhibition of calcium channels in the spinal dorsal horn, inhibition of descending serotonergic neurotransmission, and potentially also by stimulating glutamate transport activity via EAAT3 (Chincholkar 2018). Gabapentinoids have demonstrated efficacy in reducing pain in animal models of postoperative pain and inflammation; however, human studies have shown variable results with increasing side effects with higher dosages (Gottrup et al. 2004; Wallace and Schulteis 2008; Boyle et al. 2014; Chizh et al. 2007). Meta-analyses have demonstrated the strongest effectiveness for neuropathic pain following spinal cord injury (Mehta et al. 2014), no demonstrated benefit for chronic low back pain, and a high risk for adverse events (Shanthanna et al. 2017; Enke et al. 2018). Another antiepileptic medication being used for the treatment of chronic pain is lamotrigine, which blocks sodium channels. This medication is also thought to suppress the release of the excitatory neurotransmitters: glutamate and aspartate (Messenheimer 1995). The off-label use of lamotrigine for the treatment of pain is not supported by the evidence. A recent meta-analysis found no strong

supporting evidence for the use of 200–400 mg of lamotrigine daily for neuropathic pain or fibromyalgia, along with an increased risk of adverse events, including rash (Wiffen et al. 2013).

13.4.6 Modulation of Neuroimmune Cytokines

Neuroimmune modulators such as the cytokines TNF, IL-6 and IL-1β have the ability to rapidly modulate the excitatory neurotransmitter receptors AMPAR and NMDAR, as well as the inhibitory neurotransmitter receptors GlyR and GABAR. This modulation leads to enhanced excitatory neurotransmission and suppressed inhibitory neurotransmission, resulting in an amplification of pain circuits (Kawasaki et al. 2008). Thus, neuroimmune cytokines are another potential target for modulation.

Tumor necrosis factor (TNF) has been identified as a possible drug target to help lower glutamate levels in neurogenic pain (Clark and Vissel 2016). Excessive TNF can harm the nervous system by inhibiting glutaminase (the enzyme which converts non-toxic glutamine into excitatory glutamate) (Takeuchi et al. 2006) and by inhibiting glutamate reuptake (Fine et al. 1996; Zou and Crews 2005; Carmen et al. 2009). Etanercept is an anti-TNF biologic in clinical use which has shown efficacy in reducing brain glutamate levels in experimental models. However, this molecule is large enough that transport into the CNS is limited, which necessitates abnormally high doses to elicit these effects (Chio et al. 2010). Other cytokine modulating drugs are also in testing. However, many of these such as propentofylline (Sweitzer et al. 2001; Raghavendra et al. 2003), ketorolac (Wang et al. 2014), and losmapimod (Ostenfeld et al. 2013) have failed to demonstrate analgesic effects in human studies. Another IL-1 cytokine inhibitor, rilonacept, did successively show benefit in a small group of subjects suffering from gouty arthritis (Terkeltaub et al. 2009). In contrast, anakinra, an IL-1β inhibitor, failed to demonstrate beneficial effects in chronic fatigue syndrome (Roerink et al. 2017). Analgesic benefit in fibromyalgia has been successfully realized from naltrexone, which blocks microglial activation (Younger and Mackey 2009). Overall, these immune modulating agents need more research, and similar to other compounds, have been limited in the translation from animal to human research. Animal models are extremely important for testing newly developed medications. However, animal models cannot reproduce every aspect of the varying chronic pain conditions, and this may be part of the reason for the gap in translation between preclinical and clinical experiments (Yekkirala et al. 2017).

13.4.7 Non-Pharmacological Effects on Glutamatergic Neurotransmission

In addition to exploring novel pharmacological treatment options for chronic pain, it is also important to optimize non-pharmacological effects on glutamatergic neurotransmission. One of the most exciting emerging treatment options for chronic pain is through dietary modulation. The most promising dietary intervention currently being explored for the treatment of multi-symptom widespread chronic pain is through reduction of free glutamate (and aspartate) in the diet (Holton et al. 2012). Monosodium glutamate (MSG) is a well-known flavor enhancer in the diet, but other sources of free glutamate are more ubiquitous and can be hidden under many food additive names, such as hydrolyzed proteins, autolyzed yeast extract, protein concentrate, protein isolate, and others. Aspartate has the ability to activate the NMDA receptor and is also restricted on the low glutamate diet, with common sources being foods with aspartame and gelatin in them. Free glutamate is also found as a naturally occurring substance in some products like soy sauce and aged cheeses, so these are also excluded on the diet. Typically, the blood-brain barrier (BBB) limits transport of glutamate from the blood into the brain (Smith 2000), which protects an individual from high circulating plasma concentrations from dietary intake. However, it is well known that the BBB can become permeable after stress (Robinson and Moody 1980; Belova and Jonsson 1982), infection (Afonso et al. 2007) including HIV infection (McRae 2016), trauma (Barzo et al. 1996), neurotoxic exposures (Ravid et al. 2018), and as mentioned earlier, by substance P, which is released during prolonged pain transmission (Sorby-Adams et al. 2017). Thus, there are many instances where dietary intake of glutamate may become relevant for the treatment of chronic pain (and other conditions mediated by glutamate). Zanfirescu and colleagues have demonstrated that MSG administered orally to mice can reduce pain thresholds and significantly increase brain nitric oxide (NO) levels (Zanfirescu et al. 2017). This is consistent with well-known reports of sensitivity to MSG as a trigger for primary headache and migraine (Taheri 2017; Shimada et al. 2013; Baad-Hansen et al. 2010; Headache Classification Committee of the International Headache Society (IHS) 2013). Injection of MSG into the masseter muscle in humans (Shimada et al. 2015), as well as oral MSG consumption (Shimada et al. 2016), has also been shown to elicit jaw pain. My research also supports this idea, as I have demonstrated significant improvement in fibromyalgia and irritable bowel symptoms after subjects followed a low glutamate diet for one month. In this study, 84% of subjects had $\geq$30% of their symptoms remit and eight subjects had complete remission of all symptoms, after one month on the low glutamate diet. Upon double-blind placebo-controlled crossover challenge, symptoms significantly returned after challenge with MSG as compared to placebo, demonstrating that it was the restriction of glutamate from the diet, as opposed to some other inadvertent dietary effect, which caused the symptom improvement (Holton et al. 2012). Vellisca and colleagues restricted only MSG and aspartame from the diet of fibromyalgia subjects, and reported significant improvement at one month, but not at

three months. However, this study failed to remove all sources of free glutamate and aspartate from the diet, failed to monitor dietary adherence, and did not challenge subjects in a double-blind placebo-controlled manner (Vellisca and Latorre 2014). Our lab has also demonstrated significant improvement in pain impact scores after subjects in a rural village in Kenya removed free glutamate from their diets for two weeks (Holton et al. 2018). Similarly, we are currently observing profound improvements in pain and associated symptoms from treatment with a low glutamate diet in Gulf War veterans who are suffering from the multi-symptom chronic pain disorder called Gulf War Illness (GWI). The symptoms of GWI almost completely overlap with the symptoms of fibromyalgia and chronic fatigue syndrome. Significant improvements were observed after one month on the low glutamate diet in overall symptom number, myalgic score, number of tender points, and average dolorimetry across tender point sites (all $p < 0.001$). When active intervention effects were compared to wait-listed controls, the low glutamate diet demonstrated a very large effect size of $d = 1.16$, with no adverse effects reported (Holton, et al. 2020). The low glutamate diet also significantly reduced inflammatory cytokines (Holton et al. 2021) improved cognitive function (Kirkland et al. 2021), and improved depression, anxiety, and PTSD (all $p < 0.001$) (manuscript currently under review). Thus, the low glutamate diet developed in our lab may be a highly effective treatment for widespread multi-symptom chronic pain conditions, without side effects.

The low glutamate diet described above also optimizes nutrient intake, especially those nutrients with protective actions in regard to glutamate. Five nutrients stand out for their strong importance in normalizing glutamatergic neurotransmission, and include vitamin C, vitamin D, vitamin B6, magnesium, and omega-3 fatty acids.

Vitamin C (ascorbate) is best known for its role as the main water-soluble antioxidant in the diet (Carr and McCall 2017). Studies have demonstrated the profound importance of vitamin C for optimal brain function (Kocot et al. 2017), and vitamin C has been shown to be neuroprotective against glutamate excitotoxicity in animal models (Shah et al. 2015), specifically for kainate (MacGregor et al. 1996) and NMDA-related excitotoxicity (Majewska and Bell 1990; Majewska et al. 1990). Neurons in the CNS have been shown to have some of the highest concentrations of ascorbate (May 2012). Vitamin C can also directly reduce neural excitability through modulation of calcium channels (Nelson et al. 2007). Furthermore, ascorbate can also protect against the reactive oxygen species (ROS) that are produced as a result of glutamate excitotoxicity (Lane and Lawen 2009).

Vitamin D has hormonal action in the body as well as the ability to regulate gene transcription through the vitamin D responsive element (VDRE) (Nurminen et al. 2018). In-vitro studies have demonstrated that cultured cortical neurons are protected from glutamate toxicity by vitamin D, via upregulation of the vitamin D receptor on the VDRE (Taniura et al. 2006). This includes influence over the production of key enzymes and receptors which affect glutamatergic neurotransmission. For example, vitamin D can increase the gene transcription for producing the enzyme glutamic acid decarboxylase (GAD), which in turn increases the conversion of excitatory glutamate to inhibitory GABA, thereby lowering excitability in the CNS (Jiang et al. 2014). Research has demonstrated the presence of vitamin D deficiency in some

chronic pain disorders including arthritis, muscle pain, and chronic widespread pain (Wu et al. 2018), as well as the beneficial effects of vitamin D supplementation in those who were identified as having low vitamin D levels (<30 ng/dL) before starting treatment (Helde-Frankling and Bjorkhem-Bergman 2017). Furthermore, vitamin D can also affect the gene transcription for important antioxidant enzyme systems (Ferret et al. 2000) which again are needed to counteract the oxidative stress which occurs with glutamate excitotoxicity.

Vitamin B6 (as pyridoxal-5′-phosphate or PLP) functions as a cofactor for over 100 enzyme reactions, including essential action as a cofactor in the production of major neurotransmitters in the body (Brown and Beier 2020). As described earlier, glutamate is the precursor molecule for the production of GABA, the main inhibitory neurotransmitter. The enzyme used in this conversion (mentioned above), glutamic acid decarboxylase, also necessitates the use of vitamin B6 as a cofactor (Modi et al. 2015). Thus, deficiency in vitamin B6 may contribute to an imbalance in glutamate versus GABA, supporting excessive excitation in the nervous system.

The mineral magnesium also plays an important role in nervous system functioning. As mentioned earlier, it serves as a blockade to the NMDA receptor and is thought to help protect against excitotoxicity mediated by glutamate (Kirkland et al. 2018). Additionally, it has been suggested that it may positively affect the function of GABA A receptors (Moykkynen et al. 2001) creating a net inhibitory effect. Deficiency of magnesium is common (Workinger et al. 2018) and is likely to result in increased susceptibility to excitotoxicity and low GABA levels.

Omega-3 fatty acids have been shown to be beneficial for brain aging by increasing synaptic plasticity and reducing brain inflammation (Cutuli 2017), and are also thought to be able to modulate glutamatergic neurotransmission. For example, animals deficient in omega-3 fatty acids were shown to have higher brain glutamate levels in the prefrontal cortex than control animals and those fed DHA-rich diets (McNamara et al. 2017). Omega-3 fatty acids also have been shown to reduce function of astroglial glutamate transporters at basal levels, while not affecting glutamate transport during reactivity (Grintal et al. 2009). They have also been shown to decrease inflammation and increase the fluidity of cell membranes (Calder 2010), which may allow for more efficient neurotransmission. Beneficial effects of supplementation with omega-3 fatty acids have been shown for the majority of studies conducted on arthritis pain (Abdulrazaq et al. 2017), and a meta-analysis of effects on chronic pain reported a pooled random effects estimate of overall improvement from intervention studies, with a standard mean difference of −0.40 (95% CI −0.58, −0.22) for pain studies including arthritis, migraine, dysmenorrhea, chronic myalgia, and myofascial pain (Prego-Dominguez et al. 2016).

Taken all together, there is good evidence that diet can impact glutamatergic neurotransmission and pain specifically. Future research should expand on this work and directly compare the beneficial effects and side effects of dietary manipulation to current pharmacological approaches.

Two other alternative treatments which may affect glutamatergic neurotransmission include acupuncture and exercise. Animal models suggest that

electroacupuncture can reduce upregulated NMDA and AMPA receptor expression in the spinal cord of an inflammatory pain model (Choi et al. 2005), and downregulation of NMDA in the rostral ventromedial medulla in a model of visceral pain (Qi and Li 2012). Thus, electroacupuncture may be able to induce analgesia through changing expression of glutamate receptors. Similarly, exercise has been shown to increase pain thresholds and induce analgesia; however, the use of this intervention is slightly nuanced, since it also has the ability to increase pain in some conditions (Lima et al. 2017). Demonstrated benefit has been shown from high intensity swimming, which decreased glutamate-induced nociception (Martins et al. 2017). Similarly, women with fibromyalgia have experienced reduced interstitial glutamate levels in their vastus lateralis, and reduced pain levels, after exercise intervention (Gerdle et al. 2016). More research is needed to fully understand how these alternative treatments may be able to impact pain via changes to glutamatergic neurotransmission. Moreover, there may be great potential for combination therapies in the treatment of chronic pain.

13.5 Conclusion and Future Perspectives

In summary, pharmacological modulation of glutamatergic neurotransmission can effectively treat chronic pain conditions but is currently limited in its scope. Direct antagonism of the NMDA receptor is effective for some pain conditions, but is limited due to common side effects, and restrictive treatment modalities, such as the need for IV administration of ketamine for optimal effectiveness, and the high abuse potential for these types of medications. Modulation of metabotropic glutamate receptors has shown promising results in animal studies and is a continuing area of research to find effective compounds for human testing. Modulation of glutamate transporters is effective for the treatment of pain (e.g. amitriptyline) and promising work is being conducted on beta-lactam agents (ceftriaxone and clavulanic acid). Lastly, emerging research on alternative treatments such as modulating glutamatergic neurotransmission via dietary intervention suggests very promising potential for this strategy, and other treatments including electroacupuncture and exercise may also have beneficial effects on glutamatergic neurotransmission. These non-pharmacological approaches deserve further study. Moreover, it is possible that a multi-modal treatment approach may have greater benefits than a single modality for the treatment of chronic pain through the modulation of glutamatergic neurotransmission.

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Chapter 14
Pharmacological Role of Glutamate Transporters in Substance Use Disorders

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Abstract Substance use disorders (SUD) represent a public health crisis worldwide. The development of effective pharmacotherapeutics to treat drug abuse and addiction requires the identification of targetable neurobiological mechanisms. As the primary excitatory neurotransmitter in the brain glutamate possesses a significant role in plasticity, learning, and memory, and represents a promising neurotransmitter of focus for intervention in the etiology of SUDs. Chronic drug exposure induces lasting neuroadaptations in the glutamatergic system specifically within the mesocorticolimbic (MCL) reward pathway which is posited to generate maladaptive deficits in behavioral-control, thus contributing to the addictive cycle. Maintaining the strict control of glutamate release and clearance is required for homeostasis as well as the prevention of neurotoxicity and oxidative stress. There are five excitatory amino acid transporters (EAATs) and three vesicular glutamate transporters. These function to preserve homeostatic levels of glutamate under normal physiological conditions. This review aims to highlight and summarize the preclinical evidence for dysregulation of glutamate transport following drug exposure. Additionally, alterations in glutamate transporters, with an emphasis on glutamate transporter 1 (EAAT2 encodes by *SLC1A2*) and its role in the development of detrimental

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_14

drug-seeking behaviors, as well as current glutamate transporter-associated treatments being investigated are discussed.

Keywords Substance Use Disorder (SUD) · Glutamate · Excitatory amino acid transporters (EAAT) · Vesicular glutamate transporter (vGluT) · Ceftriaxone · n-acetylcysteine (NAC)

14.1 Introduction

Glutamic acid is a polar amino acid often found in an electrically charged state within the human body. The ionized form, glutamate, is the most abundant as well as the primary excitatory neurotransmitter in the mammalian central nervous system (CNS). Glutamate is directly involved in a number of biological functions including energy metabolism, cellular differentiation, protein synthesis, and synaptogenesis through activation of its distinct receptor subtypes or cellular uptake (Zhou and Danbolt 2014). Glutamate also serves as a precursor for GABA synthesis via glutamate decarboxylase (GAD) or is transferred into the TCA/Krebs Cycle as α-ketoglutarate following metabolism by glutamate dehydrogenase (Rowley et al. 2012; Bell et al. 2016b). Decades of research have demonstrated that glutamate neurotransmission is fundamental to the cellular and molecular mechanisms of synaptic plasticity and subsequent learning and memory (Kauer and Malenka 2007). Importantly, drug-induced pathological neuroadaptations to the glutamatergic system has been found to contribute significantly to the development of substance use disorders (SUDs) and other addictions (Kalivas 2009; Bell et al. 2016a; Kalivas and Volkow 2016; Scofield et al. 2016; Alasmari et al. 2018a, b). SUDs are characterized by reduced behavioral flexibility in response to drug reinforcement, which has been proposed to stem from enhanced drug-seeking behavior with simultaneous decreases in responses to non-drug stimuli (i.e., fixation; Volkow et al. 2019). Thus, integration of known changes that occur within the glutamatergic system, as well as opposing mechanisms that moderate glutamatergic signaling, following chronic drug exposure is necessary to construct accurate global models of the addiction process (Siggins et al. 2003; Basavarajappa et al. 2008; Leriche et al. 2008; Nam et al. 2012; Koob 2013; Tabakoff and Hoffman 2013). Therefore, the goal herein is to explore the mechanisms that regulate glutamate uptake and transport within the mesocorticolimbic reward neurocircuitry as it pertains to SUDs (Koob et al. 2014; Rao et al. 2015).

14.2 Glutamate & Reward Neurocircuitry

To process reward, the brain utilizes complex neurocircuitry that encompasses several nuclei, projections, and neuromodulators to integrate and evaluate responses to rewarding stimuli and direct motivational behavior accordingly. A well-established projection within this circuitry is the mesolimbic dopamine (DA) pathway (Fig. 14.1). This "reward" pathway originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (Acb) (Di Chiara and Imperato 1988; Volkow et al. 2019). A consistent observation throughout the literature is that addictive substances produce a significant elevation in DA levels within the mesolimbic pathway, thereby exerting a modulatory role on reward processing (Di Chiara and Imperato 1988; Volkow and Morales 2015). Currently, the more predominant view is that the net effect of an organism's exposure to rewarding/reinforcing stimuli is processed through both the direct and indirect actions of a drug on numerous nuclei within the CNS (Volkow et al. 2019). Neurocircuitry that functions to mediate behavioral and cognitive processes including decision making, learning, memory, emotion, and sensory processing is widespread and has been implicated to also have a role in reward processing (Bell et al. 2013; Floresco 2015; Rao et al. 2015; Koob and Volkow 2016). For instance, modulation of reward behavior by serotonin (5-HT) and norepinephrine (NE) can be traced to the dorsal (DR) as well as median (MR) raphe nuclei and the locus coeruleus (LC), respectively (Cools et al. 2011; Lisieski et al. 2019). Inhibitory influence by γ-amino butyric acid (GABA), the principal inhibitory neurotransmitter in the CNS, is released from medium spiny neurons (MSN) and interneurons throughout the reward neurocircuitry (Morales and Margolis 2017; Seo et al. 2016; Yang et al. 2018). Modulatory actions via glutamate is ubiquitous and occurs at several levels of reward processing (cf., Floresco et al. 2001, 2003; Bell et al. 2012, 2013, 2016b, 2017, 2019; Morales and Margolis 2017). Moreover, it has become increasingly clear that interactions between DAergic and glutamatergic systems within the "reward" neurocircuit play a major role in addiction (Schmidt and Reith 2005). Thus, glutamate plays an integral role in reward/reinforcement processing that mediates addiction.

The mesocorticolimbic (MCL) system encompasses several cortical and limbic brain structures with several projections which have been strongly implicated in addiction. Central to this system is the VTA which is primarily composed of DA neurons that project to the Acb (mesolimbic) and the prefrontal cortex (PFC; mesocortical) and, to a lesser extent, the amygdala (Amyg) and hippocampus (HPC; extended Amyg; McBride 2002; Morales and Margolis 2017). Activity in both pathways is heavily modulated by glutamatergic signaling which, under normal circumstances, maintains a state of glutamate homeostasis (Scofield et al. 2016). Structures including the PFC, basolateral amygdala (BLA), HPC, and paraventricular nucleus of the thalamus (PVN) provide glutamate innervation to the MCL and act to modulate neural activity associated with reward as well as reinforcement (Fig. 14.1; Wassum and Izquierdo 2015; Cooper et al. 2017; Bossong

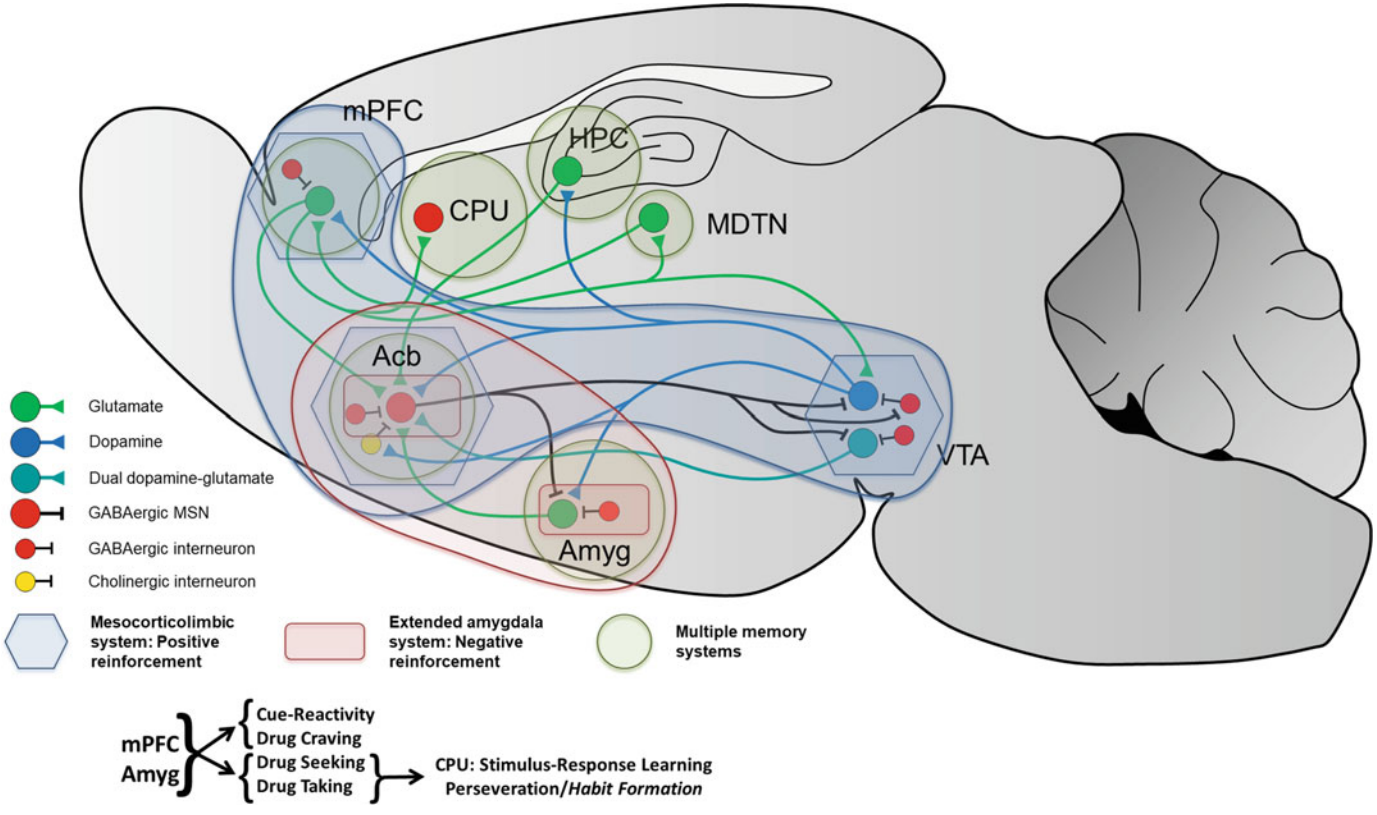

Fig. 14.1 Mesocorticolimbic reward circuitry. Simplified diagram of the ventral tegmental area (VTA) and nucleus accumbens (Acb) reward circuit. The illustration depicts key neuronal projections within the mesocorticolimbic system implicated in drug-related learning, reward, memory, and abuse. The primary reward circuit includes projections from the VTA to the Acb, which releases dopamine in response to reward stimuli. GABAergic projections from the Acb to the VTA occur through a direct pathway mediated by D1-type medium spiny neurons (MSNs) that innervate the VTA or the indirect pathway mediated by

D2-type MSNs which innervate the VTA via GABAergic neurons in the ventral pallidum (not shown). The Acb receives dense glutamatergic inputs from the hippocampus (HPC), basolateral amygdala (BLA), paraventricular nucleus of the thalamus (PVN), and medial prefrontal cortex (mPFC). These glutamatergic inputs control aspects of reward-related perception and memory. Additionally, modulation of reward circuitry occurs via serotonin and norepinephrine systems from the dorsal raphe nuclei (DR) and locus coeruleus (LC), respectively. CPU, caudate putamen; MDTN, medial dorsal thalamic nucleus. The multiple memory systems are depicted by the green circles and include: HPC-mediated spatial and autobiographical learning and memory; CPU-mediated automatic/stimulus-response learning and memory; amygdala-mediated fear-associated learning and memory as well as salience modulation of HPC- and CPU-mediated learning and memory; Acb-mediated conditioned place preference, which is also modulated by input from the amygdala; and PFC-mediated working memory

et al. 2018; Otis et al. 2019). The Acb is divided into the shell (AcbSh) and core (AcbCo) subregions which receive glutamatergic innervation from the infralimbic (IL) and prelimbic (PL) regions of the medial mPFC, respectively (Kelley 1999; McBride et al. 1999) and exhibit opposing influence on motivated behavior associated with reward (i.e., PL→AcbC = go; IL→AcbSh = stop; Peters et al. 2009; Gass and Chandler 2013; Gourley and Taylor 2016). Thus, the Acb represents an important point of convergence for reward signaling that is heavily influenced by MCL-associated glutamate projections (Fig. 14.1; Di Chiara and Imperato 1988; Floresco 2015; Scofield et al. 2016).

14.3 Glutamate Regulation & Trafficking

Glutamate synthesis and metabolism is cyclical in nature. The metabolic, diffusion, transport, and catabolic processes significantly contribute to the maintenance of glutamate homeostasis and the prevention of neuronal excitotoxicity that can result from excessive synaptic glutamate and subsequent overactivation of glutamate receptors. The concentration of glutamate is strictly controlled, with basal levels varying considerably across nuclei and neurocircuits. Intracellular glutamate concentration is the greatest within synaptic vesicles where it can reach 100 mM (Hayashi 2018). Other intracellular glutamate levels are estimated to be near 2 mM, while extracellular levels are in the low micromolar range. Glutamate in the synaptic cleft is maintained at an even lower level at less than 20 nM during resting conditions which can briefly exceed 1 mM following action potential mediated release (Moussawi et al. 2011; Hayashi 2018; Mahmoud et al. 2019). Glutamate returns to resting levels within milliseconds through both diffusion and transport. The subregional differences in concentration gradients within the CNS indicate the importance of maintaining normal physiological levels both temporally and spatially as well as its potential role in neuropsychiatric diseases (Kalivas 2009; Bell et al. 2016a; Spencer et al. 2016).

In contrast to many neurotransmitters that rely heavily on neuronal uptake, glutamate uptake regulation is highly dependent upon glial cells (i.e., astrocytes). Glial regulation occurs via active transport of glutamate from the synapse into surrounding astrocytes that is then converted into glutamine by glutamine synthetase (GS; Fig. 14.2; Danbolt 2001; Zhou and Danbolt 2014; Logica et al. 2016). Next, the newly synthesized glutamine is shuttled from astrocytes back to neurons via glutamine transporters (GlnT) found in the plasma membrane of both cell types (Fig. 14.2). Specifically, GlnTs are members of the sodium-coupled neutral amino acid transporter (SNAT) family and utilize the electrochemical gradient across membranes to transport against concentration gradient. These include SNAT3 (*SLC38A3*) and SNAT5 (*SLC38A5*), which move glutamine out of the glial cell and into the peri-synapse where concentrations range from 200 to 800 μM (Bröer and Brookes 2001; Pochini et al. 2014). Glutamine is then transported into the excitatory presynaptic compartment at concentrations up to 20 mM through

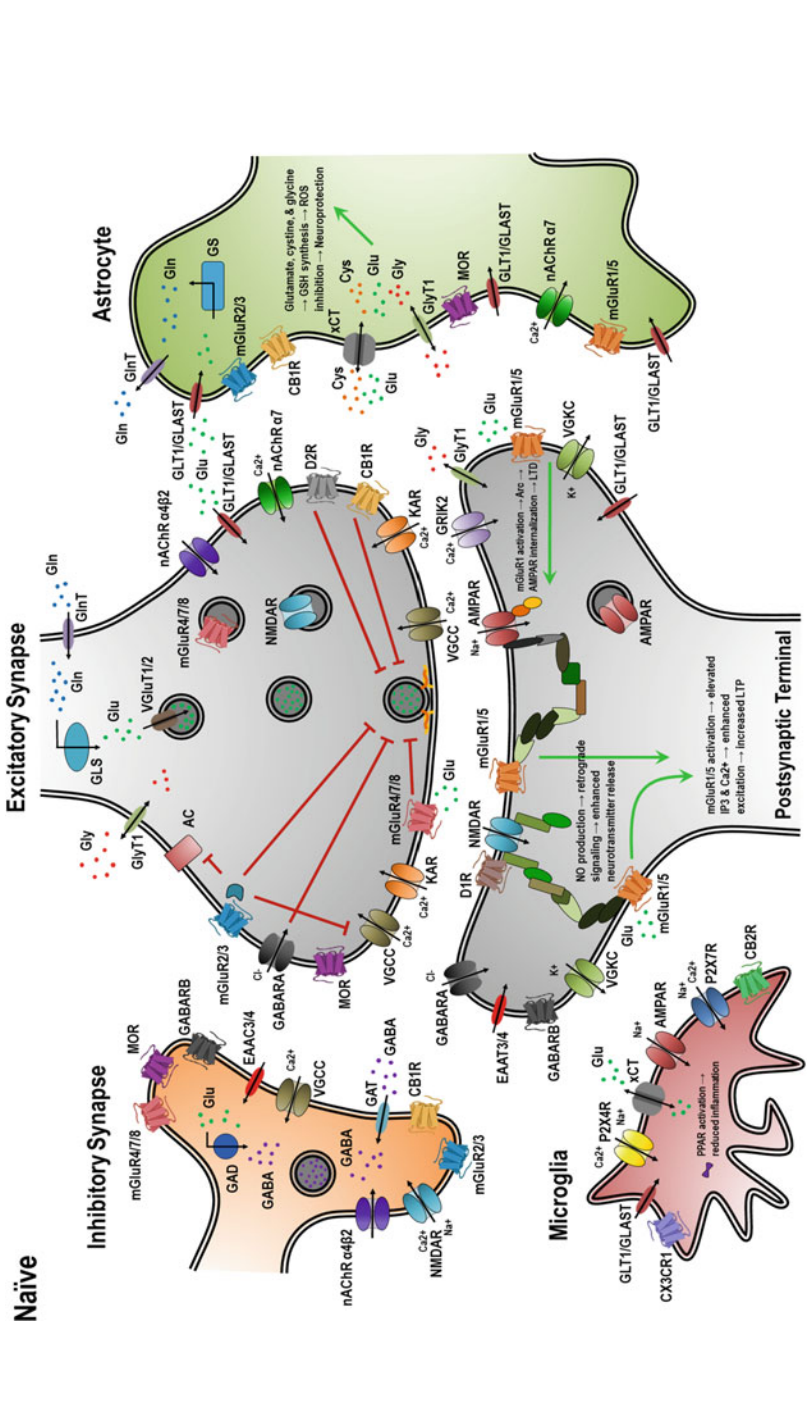

Fig. 14.2 Naïve excitatory synapse. Simplified illustration of a prototypic glutamatergic synapse in the brain. Functionally, Inhibitory control over excitatory neurotransmission is predominantly mediated through the release of GABA to maintain normal CNS function; and, microglia are the resident immune cells of the brain with activity levels and morphology that are highly dependent on physiological or pathological influences. Microglia in the surveillance state, previously referred to as resting state, are mobile cells that continuously monitor the surrounding microenvironment for immune signals. Once activated, microglia functioning can range from pro-inflammatory to neuroprotective based on the initiating neuroimmune factors. The presynaptic terminal governs glutamate release through a number of mechanisms. Under normal conditions, the reliability and magnitude of glutamate release can be reduced through stimulation of presynaptically located Gi/o coupled G-protein coupled receptors (GPCR) such as group II metabotropic glutamate receptors (mGluR2/3),

Fig. 14.2 (continued) dopamine receptor D2 (D2R), cannabinoid 1 receptors (CB1R), or mu-opioid receptors (MOR). Conversely, glutamate release can be enhanced by increased afferent stimulation through activation of presynaptic Gs coupled GPCRs, nicotinic acetylcholine receptors (nAChR), or retrograde messengers released from the postsynaptic cell. Postsynaptic terminals are highly dynamic and responsive to changes in *N*-methyl-*D*-aspartate receptor (NMDAR), voltage-gated calcium channels (VGCC), group I metabotropic glutamate receptors (mGluR1/5), and postsynaptic calcium transient activity. Additionally, the postsynaptic density (PSD) is a cytoskeletal specialization located in the postsynaptic terminal that contains integral receptors, scaffolding proteins, kinases, and other secondary messengers critical for downstream signal integration and synaptic plasticity. Finally, astrocytes play a central role in regulating glutamatergic transmission. Maintenance of homeostasis occurs through rapid buffering of potassium and glutamate, supplying neuronal glutamine (Gln) by its conversion from glutamate via the enzyme glutamine synthetase (GS), and generation of a primary CNS antioxidant glutathione (GSH)

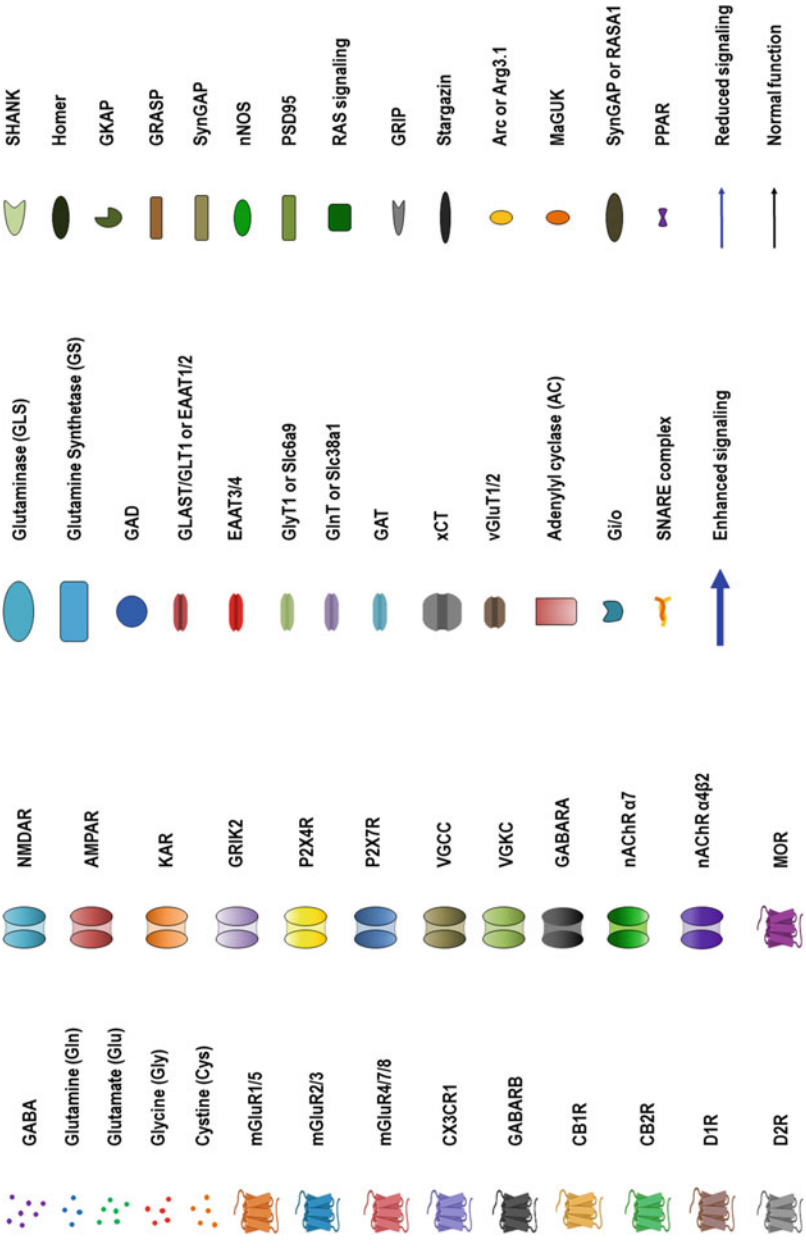

Fig. 14.2 (continued)

Table 14.1 Summary of glutamate transporters

Excitatory Amino Acid Transporters (EAAT)					
Human	Rodent	Gene	CNS distribution	Cell type	Subcellular localization
EAAT1	GLAST	SLC1A3	cerebral cortex, cerebellum, spinal cord	Astrocytes, oligodendrocytes	perisynaptic
EAAT2	GLT-1	SLC1A2	whole brain, cerebellum, spinal cord, retina	astrocytes, neurons	perisynaptic, presynaptic
EAAT3	EAAC1	SLC1A1	hippocampus, striatum, cerebellum	predominantly neurons, some glia	postsynaptic, cell soma, dendrites
EAAT4	EAAT4	SLC1A6	cerebellum	Purkinje cells	postsynaptic, dendrites
EAAT5	EAAT5	SLC1A7	retina	bipolar cells, photoreceptors	presynaptic
Vesicular Glutamate Transporters (vGluT)					
vGluT1	vGluT1	SLC17A7	cerebral cortex, cerebellum, spinal cord	glutamatergic neurons, astrocytes	synaptic vesicles, axon terminals
vGluT2	vGluT2	SLC17A6	ventral tegmental area, basolateral amygdala, nucleus accumbens, brain stem	glutamatergic neurons, dopaminergic neurons	synaptic vesicles, axon terminals
vGluT3	vGluT3	SLC17A8	hippocampus, nucleus accumbens, dorsal striatum, olfactory tubercle, medial raphe nuclei	serotonergic neurons, acetylcholinergic neurons, GABA interneurons, glutamatergic neurons, and astrocytes	synaptic vesicles, cell soma, dendrites, glial endfeet

SNAT1 (*SLC38A1*), SNAT2 (*SLC38A2*), and/or SNAT7 (*SLC38A7*; Chaudhry et al. 2002a, b). Importantly, glutamine can be moved in and out of the synaptic space without inducing neurotoxic cascades (Deitmer et al. 2003; Pochini et al. 2014; Zhou and Danbolt 2014; Rao et al. 2015). This metabolic/catabolic sequence is particularly advantageous in that it reduces excessive synaptic glutamate levels which can produce neuroadaptations associated with SUDs and neurotoxicity (Aschner et al. 2007; Lan et al. 2014). Glutaminase then converts intraneuronal glutamine into glutamate (Rowley et al. 2012), which is packaged into secretory vesicles by vesicular glutamate transporters (vGluT) in preparation for exocytosis. These include vGluT1 (*SLC17A7*), vGluT2 (*SLC17A6*), and vGluT3 (*SLC17A8*; Table 14.1; Bellocchio et al. 2000; Takamori et al. 2000a, b).

There is a significant potential for excessive glutamate in the synapse to induce overactivation of receptors leading to excitotoxicity and neuronal death. Thus, efficient glutamate uptake and transport from the synapse and surrounding area is essential to prevent cell death (Danbolt 2001; Rao et al. 2015; Bell et al. 2016a;

Mahmoud et al. 2019; Zhang et al. 2019). There are five transporters that regulate extracellular glutamate levels and these are part of the solute carrier 1 (*SLC1A*) family. These transporters are excitatory amino acid transporters (i.e., EAAT1, EAAT2, EAAT3, EAAT4, and EAAT5) and correspond to human genes *SLC1A3*, *SLC1A2*, *SLC1A1*, *SLC1A6*, and *SLC1A7*, respectively (Table 14.1). The rodent homologues are referred to as glutamate aspartate transporter (GLAST; *Slc1a3*), glutamate transporter 1 (GLT-1; *Slc1a2*), excitatory amino acid carrier 1 (EAAC1; *Slc1a1*), EAAT4 (*Slc1a6*), and EAAT5 (*Slc2a7*; Wadiche et al. 1995; Arriza et al. 1997; Tanaka 2000). Similar to GlnTs, EAAT makes use of electrochemical gradients to transport glutamate against its concentration gradient. This occurs through cotransport of one H^+ and three Na^+ ions along with the glutamate molecule while exporting a single K^+ ion (Grewer et al. 2008).

Glycine and glycine transport are also critical when exploring the prototypical excitatory synapse. The *N*-methyl-D-aspartate receptor (NMDAR) contains subunits with a co-agonist glycine binding site that potentiates glutamate signaling as well as priming the receptor for internalization (Nong et al. 2003). Glycine transporter 1 (GlyT1) encoded by *SLC6A9* is principally localized on glia, while GlyT2 (*SLC6A5*) is neuronally expressed at excitatory synapses. Additionally, there has been increased interest in the efficacy of N-acetylcysteine to treat neuropsychiatric disorders. It is therefore equally important to recognize the significance of the cystine–glutamate exchanger (xCT; *Slc7a11*) and its effects on reversing neuronal damage induced by excitotoxicity and/or oxidative stress (Lewerenz et al. 2013). The xCT is commonly localized on astroglial cells and functions to exchange extracellular cystine for intracellular glutamate at a one-to-one ratio (Watts et al. 2014). Glutamate is released in the exchange of cystine and binds at the presynaptic mGluR2/3, thereby blocking synaptic glutamate release (Javitt et al. 2011; Moran et al. 2005) and acting as a regulatory mechanism of glutamate homeostasis. Next, cystine can be converted into cysteine, which is used to synthesize glutathione as well as other proteins. Glutathione is a key antioxidant and functions to prevent or reverse neuronal injury induced by excessive levels of glutamate and free radicals (Patten et al. 2013).

14.4 Vesicular Glutamate Transporters

The vesicular glutamate transporters (vGluTs) are highly expressed in neurons throughout the CNS with vGluT1 and vGluT2 more commonly found in glutamatergic cells (Table 14.1). Specifically, vGluT1 localization is generally widespread and found in the HPC, Amyg, Acb, PFC, cerebellum, and spinal cord. Expression of vGluT2 is more limited and is localized to the BLA, Acb, and VTA. On the other hand, vGluT3 is found primarily in non-glutamatergic cells (e.g., serotonergic, glial, GABAergic, cholinergic) of the Acb, olfactory tubercle, HPC, and MRN (Wang et al. 2019; Zhang et al. 2019). Relative to EAATs, vGluTs display 100–1000-fold less affinity for glutamate (Shigeri et al. 2004). Importantly, vGluTs

have a micromolar affinity for glutamate but do not transport aspartate, glutamine, or GABA. The function of vGluTs is known to be dependent upon a vesicular proton electrochemical gradient that is produced by ATPase activity. The transporters also have a biphasic interaction with Cl^-, where low concentrations initiate uptake while higher concentrations have an inhibitory action on transporter function (Shigeri et al. 2004).

Alterations in vGluT1 have been associated with schizophrenia, addiction, Alzheimer's disease, and epilepsy (Alonso-Nanclares and De Felipe 2005; Eastwood and Harrison 2005; Mark et al. 2007; van der Hel et al. 2009). For example, vGluT1 mRNA was increased five-fold in the DRN of rats following peri-adolescent binge like alcohol drinking. This change was coupled with a significant reduction in both vGluT2 and vGluT3 mRNA expression levels (McClintick et al. 2015). Additionally, following exposure to methamphetamine there was a significant and long-lasting increase in vGluT1 mRNA and protein levels in the striatum (Mark et al. 2007). Knackstedt and colleagues (2009, 2010) reported a reduction in vGluT1 expression in the AcbCo following self-administration of cocaine or nicotine. Due to the distinct regional and cellular expression of vGluT isoforms, these proteins are often used as markers to delineate specific neuronal subpopulations. The deletion of vGluT2 induced prenatal or neonatal mortality and an almost complete loss of glutamate activity in the thalamus, but not in the HPC (Moechars et al. 2006). Activation of vGluT2 expressing DA neurons in the VTA enhanced learning of a conditioned place preference as well as reinforcing instrumental behavior (Wang et al. 2015). Repeated deprivations from alcohol reduced vGluT2 in the AcbSh (Zhou et al. 2006). The involvement of vGluT3 is involved in fear, stress, hearing, as well as stimulant-induced locomotor activity (Ryu et al. 2017; Balazsfi et al. 2018; Li et al. 2018; Mansouri-Guilani et al. 2019; Sakae et al. 2019). Collectively, these findings provide evidence that vGluTs may play an important role in addiction behaviors.

14.5 Plasma Membrane Glutamate Transporters

Glutamate transporters are located throughout the brain. EAAT1, or GLAST, is located both on the plasma membrane and the mitochondrial membrane of glial cells (i.e., astrocytes, microglia, and oligodendrocytes). EAAT2 (GLT-1) is located on astrocytes, microglia, oligodendrocytes and on axon terminals (e.g., CA3 of the HPC) and represents the primary transporter that removes more than 90% of glutamate from the synapse, which is necessary to prevent excitotoxicity and promote normal physiological function (Danbolt 2001). EAAT3, encoded by *SLC1A1*, is located on neurons, specifically dendrites and axon terminals. Like the predominantly glial transporters, EAAT3 removes excess glutamate from the synapse but also transports aspartate and cysteine. A *SLC1A1* polymorphism is present in a subpopulation of individuals with obsessive-compulsive disorder (Stewart et al. 2013). In addition, there is some evidence that amphetamine leads to internalization

of EAAT3 and this may coincide with internalization of the DA transporter as well (Underhill et al. 2014). EAAT4 is expressed predominantly in the cerebellum transporting both glutamate and aspartate concurrent with the transport of chloride ions (Fairman et al. 1995), as well as in spinal cord, forebrain, and astrocyte (Hu et al. 2003). In addition, the xCT (*SLC7A11*), a chloride-dependent, sodium-independent transporter is located primarily on astrocytes (Bridges et al. 2001; Lin et al. 2016). While the xCT is present throughout the brain, there is especially high expression in the BLA and PFC of the MCL (Bridges et al. 2012). Finally, the EAAT5 is found only in the retina (Table 14.1). For more information, there are additional reports that expand on the mechanisms of glutamate transport (Rothstein et al. 1994; Lehre et al. 1995; Wadiche et al. 1995; Arriza et al. 1997; Tanaka 2000; Danbolt 2001; Huggett et al. 2002; Beschorner et al. 2007; Bellesi and Conti 2010; Reissner and Kalivas 2010; Carbone et al. 2012; Karki et al. 2015; Bell et al. 2016a; Spencer et al. 2016; Mazaud et al. 2019).

14.6 Upregulating Glutamate Transporters and the Treatment of SUDs

Substantial evidence suggests that the development of substance dependence involves changes in many aspects of glutamate homeostasis. Glutamate transmission is heavily regulated by the glutamate transporters described in this review. Importantly, GLT-1 is considered the primary glutamate transporter in the brain that regulates up to 90% of extracellular glutamate. Concurrently, xCT regulates glutamate uptake through the exchange of extracellular cystine for intracellular glutamate (Bannai and Ishii 1982; Bannai 1984; Sari 2013). Modulation of glutamate transport through upregulation of GLT-1 is a promising avenue to treat dependence on drugs of abuse, including ethanol and cocaine (Rao et al. 2015; Spencer and Kalivas 2017; Alasmari et al. 2018a, b). Discussed here are the effects of medications, known to upregulate GLT-1, on the attenuation of drug-seeking behaviors. An emphasis on the use of β-lactam antibiotics, particularly ceftriaxone and N-acetylcysteine, as GLT-1 upregulators to attenuate drug-seeking behaviors is of particular interest.

14.7 Ceftriaxone and Ethanol

The expression of GLT-1 and its function can be upregulated by FDA-approved β-lactam antibiotics, which increase glutamate uptake (Rothstein et al. 2005; Spencer and Kalivas 2017). Ceftriaxone is a beta-lactam antibiotic that is known to increase glutamate reuptake through the upregulation of glial GLT-1 expression and/or function (Rothstein et al. 2005). Ceftriaxone decreases ethanol consumption and ethanol preference over water in alcohol-preferring (P) rats (Sari et al. 2011,

2013b; Rao and Sari 2014; Das et al. 2015) and outbred rats (Stennett et al. 2017). These decreases in ethanol intake are associated with normalization (i.e., reversal of ethanol-induced decreases) of GLT-1 and/or xCT protein levels in the Acb and/or PFC (Sari et al. 2011, 2013a, 2013b; Rao and Sari 2014; Das et al. 2015). Ceftriaxone attenuated ethanol-induced increases in extracellular glutamate in the Acb in male P rats (Das et al. 2015), an effect that is likely mediated through upregulation of GLT-1. In contrast, Stennett et al. (2017) found that ethanol intake in Sprague-Dawley rats did not alter GLT-1 and xCT protein levels, which suggests that there might be dysfunction of these transporters without alteration of their expression. However, Sprague-Dawley rats consume much less ethanol than Wistars, Long-Evans, and selectively bred alcohol-preferring rat lines (cf., Bell et al. 2014) possibly leading to a floor-effect in the Stennett et al.' (2017) study. It is important to note that the expression of GLT-1 was not affected in the PFC and Acb in P rats that were experiencing relapse-like ethanol behavior (Qrunfleh et al. 2013). However, ceftriaxone treatment upregulated GLT-1 in these brain regions and attenuated relapse-like ethanol-seeking behavior, which suggests that restoring dysfunctional GLT-1 is critical in the attenuation of ethanol seeking (Qrunfleh et al. 2013). Other studies confirmed the efficacy of ceftriaxone on reducing relapse-like ethanol-seeking behaviors (Abulseoud et al. 2014; Alhaddad et al. 2014b; Rao and Sari 2014) and alleviating ethanol withdrawal symptoms in male P rats (Abulseoud et al. 2014), and this effect was associated with an upregulation of GLT-1 and xCT in the Acb, PFC, and/or whole striatum (i.e., Acb, caudate, and putamen; Abulseoud et al. 2014; Alhaddad et al. 2014b) and specific upregulation of GLT-1 isoforms (GLT-1a and GLT-1b; Alhaddad et al. 2014a). Additionally, pretreatment with ceftriaxone during acquisition of ethanol drinking reduces the maintenance of ethanol intake in female adolescent and adult P rats, with a greater effect in adult rats (Sari et al. 2013a).

14.8 Ceftriaxone and Psychostimulants

Ceftriaxone appears to be more effective in reducing cocaine-seeking behaviors than cocaine self-administration itself (Sari et al. 2009; Sondheimer and Knackstedt 2011; Roberts-Wolfe and Kalivas 2015). Ceftriaxone attenuated cocaine-primed, context-induced, or other cue-induced reinstatement of cocaine-seeking behaviors (Sari et al. 2009; Knackstedt et al. 2010; Roberts-Wolfe and Kalivas 2015; LaCrosse et al. 2016; Bechard et al. 2018; Bechard and Knackstedt 2019). Ceftriaxone-induced attenuation of cocaine-seeking is associated with normalization (i.e., reversal of cocaine-induced reductions) of GLT-1 and/or xCT expression in the Acb (Kalivas 2009; Sari et al. 2009; Knackstedt et al. 2010; Sondheimer and Knackstedt 2011; LaCrosse et al. 2016; Spencer and Kalivas 2017; Bechard et al. 2018).

Importantly, ceftriaxone has also been found to attenuate reinstatement to methamphetamine seeking behavior in conditioned place preference paradigm (Abulseoud et al. 2012), possibly through overexpression of GLT-1. For instance, overexpression of GLT-1 in Acb using gene transfer technology blocked

methamphetamine reinstatement in conditioned place preference (Fujio et al. 2005). It is important to note that exposure to methamphetamine can lead to increase of glutamate release in the Acb and PFC (Ito et al. 2006; Labarca et al. 1995; Shoblock et al. 2003; Stephans and Yamamoto 1995; Xue et al. 1996). These studies would suggest that upregulation of GLT-1 with ceftriaxone is critical to the regulation of glutamate uptake and subsequent attenuation of the reinstatement of methamphetamine seeking behavior. Acute repeated exposure to high dose of methamphetamine of 10 mg/kg, i.p., every 2 h × 4/day downregulated the expression of GLT-1 in the dorsal striatum, medial PFC and Acb (Alshehri et al. 2017; Althobaiti et al. 2016b). Importantly, ceftriaxone attenuated the effects of methamphetamine-induced GLT-1 downregulation in these brain regions (Alshehri et al. 2017; Althobaiti et al. 2016b) as well as methamphetamine-induced alterations in tissue content of several neurotransmitters, including glutamate (Althobaiti et al. 2016a).

14.9 Ceftriaxone and Other SUDs

As with ethanol, cocaine, and methamphetamine, chronic nicotine exposure downregulated astrocytic GLT-1 and xCT within the Acb and/or VTA (Knackstedt et al. 2009; Gipson et al. 2013; Spencer and Kalivas 2017). However, ceftriaxone had no effect on the development of a nicotine conditioned place preference in mice (Alajaji et al. 2013), but did attenuate nicotine-induced reinstatement in conditioned placed preference paradigm (Alajaji et al. 2013; Philogene-Khalid et al. 2017) and reversed nicotine withdrawal signs (Alajaji et al. 2013). In rats, ceftriaxone reduced oral nicotine-sucrose and nicotine-ethanol intake by P rats, which was concurrent with normalization of GLT-1 expression levels in the Acb and PFC (Sari et al. 2016). Overexpression of GLT-1 in the Acb reduced morphine conditioned place preference but did not affect somatic signs of naloxone-precipitated morphine withdrawal (Fujio et al. 2005). Administration of ceftriaxone also attenuated the development of tolerance to the anti-nociceptive effect of morphine and reduced naloxone- or naltrexone-precipitated morphine withdrawal in mice and rats (Rawls et al. 2010; Habibi-Asl et al. 2014; Medrano et al. 2015). Moreover, morphine-induced conditioned place preference and morphine-associated locomotor sensitization were attenuated by ceftriaxone treatment (Schroeder et al. 2014). Shen et al. (2014) reported that heroin self-administration impaired functional glutamate uptake and decreased GLT-1 expression in the Acb. These authors also reported that ceftriaxone reduced cue-induced reinstatement of heroin seeking (Shen et al. 2014). In addition, ceftriaxone treatment attenuated morphine-induced hyperthermia (Rawls et al. 2007). A more recent study showed that ceftriaxone attenuated the reinstatement of hydrocodone-induced conditioned place preference and normalized a hydrocodone-induced reduction of xCT expression in the Acb (Alshehri et al. 2018).

14.10 Other Upregulators of GLT-1 and SUDs

Administration of the β-lactam antibiotics amoxicillin, Augmentin (amoxicillin/clavulanate; Goodwani et al. 2015; Hakami et al. 2016), and ampicillin (Alasmari et al. 2015; Rao et al. 2015) attenuates ethanol intake in male P rats. Similar to ceftriaxone, systemic administration of Augmentin and amoxicillin upregulated/normalized xCT and GLT-1 levels in the Acb and/or PFC (Alasmari et al. 2015; Goodwani et al. 2015; Hakami et al. 2016, 2017). A recent report by Hammad et al. (2017) examined the effects of the β-lactam antibiotic ampicillin/sulbactam on cocaine reinstatement by male P rats. These authors found that cocaine-primed reinstatement downregulated GLT-1 and xCT in the AcbSh and AcbCo, but not the dorsal medial PFC (dmPFC; Hammad et al. 2017). Ampicillin/sulbactam reduced cocaine-induced reinstatement in a conditioned place preference paradigm while normalizing the expression of GLT-1 and xCT in the AcbSh, AcbCo, and dorsal mPFC as well as mGluR1 levels in the AcbCo, although there was a decrease in locomotor activity following treatment (Hammad et al. 2017). Importantly, ampicillin/sulbactam attenuated cocaine-induced ethanol deprivation effects, and this effect was associated with upregulation of GLT-1 and xCT expression in the AcbSh and AcbCo as well as dmPFC (Hammad and Sari 2020).

Cefazolin and cefoperazone, both β-lactam antibiotics, decreased ethanol but not sucrose intake (Rao et al. 2015; Alasmari et al. 2016). Cefazolin and cefoperazone both upregulate GLT-1 and its isoforms (GLT-1a and GLT-1b) in the Acb and PFC (Rao et al. 2015; Alasmari et al. 2016). Regarding xCT, cefazolin increased expression in both the Acb and PFC, while cefoperazone only upregulated xCT expression in the Acb (Alasmari et al. 2016). Clavulanic acid, a β-lactamase inhibitor, upregulates GLT-1 in the Acb (Kim et al. 2016). Clavulanic acid decreased ethanol intake at a dose that was approximately 30-fold lower than ceftriaxone in P rats (Hakami and Sari 2017; Althobaiti et al. 2019). This effect was associated with restored expression of GLT-1 and xCT in Acb (Hakami and Sari 2017; Althobaiti et al. 2019) and increased the expression of mGlu2/3R in the AcbSh and mPFC (Althobaiti et al. 2019). In addition, clavulanic acid blocked the reinstatement of methamphetamine-induced condition place preference (Althobaiti et al. 2019) and this effect was associated with restoration of GLT-1 and xCT levels in the AcbSh, but not in the AcbCo. In Mice, clavulanic acid produced significantly lower breakpoints for cocaine maintained on a progressive ratio schedule of reinforcement (Kim et al. 2016). Clavulanic acid also attenuated reinstatement to morphine in rats tested using the conditioned place preference paradigm (Schroeder et al. 2014).

Other non-antibiotic drugs have been tested in male P rats and found to attenuate ethanol intake, an effect associated with upregulation/activation of GLT-1. Among these synthetic drugs, 3-(3-pyridyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate (GPI-1046), an analog of FK506, and (R)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153). GPI-1046 treatment reduced ethanol intake in P male rats and upregulated the expression of GLT-1 levels in key central reward brain regions (i.e., Acb and PFC; Sari and Sreemantula 2012).

MS-153 treatment also reduced ethanol intake and attenuated an ethanol-induced reduction in the expression of GLT-1 in the Acb, Amyg, and HPC (Aal-Aaboda et al. 2015; Alhaddad et al. 2014b).

14.11 N-acetylcysteine

N-acetylcysteine (NAC) is an FDA-approved treatment for paracetamol (acetaminophen) overdose. NAC is oxidized into cystine leading to increase in availability of cystine for the astroglial xCT (Nocito Echevarria et al. 2017). Increased levels of cystine lead to an enhancement of glutamate exchange by astroglial cells resulting in elevated concentrations of glutamate within the extrasynaptic space, increased synthesis of glutathione (GSH) in astrocytes, and restoration of downregulated GLT-1 expression (Berk et al. 2013; Brown et al. 2013; Nocito Echevarria et al. 2017). We suggest that the restoration of GLT-1 is associated with decrease in extracellular glutamate concentrations in the brain and increases in the exchange of cystine and glutamate thereby leading to increases in the biosynthesis of GSH. This is an important process to reduce oxidative stress, which might be caused with chronic exposure to drugs of abuse. Substantial research has shown that NAC has antioxidant, anti-inflammatory, and neuroprotective properties (cf., Santus et al. 2014; Shahripour et al. 2014; Bhatti et al. 2017; Markoutsa and Xu 2017; Pei et al. 2018).

14.12 N-acetylcysteine and Ethanol

Oral administration of NAC reduced ethanol intake, relapse drinking, and relapse-associated blood ethanol concentrations in the Wistar derived University of Chile Bibulous (UChB) alcohol-preferring rats (Quintanilla et al. 2016, 2018; Israel et al. 2019). Additionally, NAC fully abolished increased levels of oxidative stress and the neuroinflammation induced by chronic ethanol intake by UChB rats (Quintanilla et al. 2018). NAC administration in an ethanol-dependent animal model reduced ethanol-intake, operant ethanol-self-administration, ethanol break-point (i.e., progressive ratio), ethanol-seeking behavior, and relapse-like ethanol-seeking behavior (Lebourgeois et al. 2019). Moreover, NAC prevented stress-potentiated ethanol intake and abolished conditioned stress-induced reinstatement of ethanol-seeking behavior in outbred rats (Garcia-Keller et al. 2019).

14.13 N-acetylcysteine and Cocaine

NAC appears to have limited effects on cocaine self-administration as it failed to alter cocaine self-administration in rats (Murray et al. 2012; Frankowska et al. 2014) or non-human primates (Kangas et al. 2019). Nevertheless, it appears to be intricately involved in drug learning as others have reported that NAC prevented cocaine-primed (Baker et al. 2003; Amen et al. 2011; Frankowska et al. 2014), and cue-induced (Reichel et al. 2011; Murray et al. 2012; Frankowska et al. 2014; Reissner et al. 2015) as well as stress-induced (Garcia-Keller et al. 2019), reinstatement of cocaine-seeking in rats but not in non-human primates (Kangas et al. 2019). NAC has also been found to facilitate extinction of drug-lever responding in rats (LaRowe and Kalivas 2010) and non-human primates (Kangas et al. 2019). In addition, Murray et al. (2012) reported that NAC was able to attenuate both early and late stages of acquisition and maintenance of cue-induced cocaine-seeking behavior. Intra-accumbal NAC attenuated cue-induced cocaine-seeking behavior and cue-cocaine primed reinstatement of cocaine-seeking behavior, which was enhanced by the mGluR5 antagonist MTEP (Kupchik et al. 2012). NAC restored the expression of GLT-1, but not xCT, in MCL subregions, which was critically important for the ability of NAC to suppress cue-induced reinstatement of cocaine-seeking behavior (Reissner et al. 2015; Ducret et al. 2016). Another study reported that NAC prevented the loss of control observed with chronic cocaine self-administration (Madayag et al. 2007). However, in other work acute, chronic, and progressive-ratio cocaine self-administration was not affected by NAC, although NAC did facilitate punishment-induced extinction (Ducret et al. 2016). The discrepancy between these studies may be due to differences in cocaine training history, the dose of cocaine used, or timing of NAC administration prior to drug availability or exposure among other experimental procedures.

14.14 N-acetylcysteine and Other SUDs

Acute administration of NAC can decrease nicotine self-administration without altering food self-administration, whereas chronic administration lasting 14 days had a non-specific attenuating effect on both nicotine and food self-administration (Ramirez-Niño et al. 2013). Furthermore, acute NAC attenuated cue-induced reinstatement of nicotine-seeking behaviors (Ramirez-Niño et al. 2013). Subchronic NAC administration for five days produced mixed results on cue-induced nicotine-seeking. One study found that this regimen of NAC exposure reduced cue-induced nicotine-seeking in male Sprague-Dawley rats but not female rats regardless of estrous cycle phase (Goenaga et al. 2020), while another study found that 5 days of NAC treatment did not alter cue-induced nicotine-seeking in male Sprague-Dawley rats (Powell et al. 2019). These results suggest that there may be sex specific effects of NAC with regard to nicotine craving/relapse behaviors (Goenaga et al.

2020) although the studies did possess differences in experimental procedures which may have affected the results.

Chronic administration of NAC for 14–15 days has consistently inhibited cue-induced nicotine-seeking behavior (Ramirez-Niño et al. 2013; Moro et al. 2019; Namba et al. 2019; Powell et al. 2019; Goenaga et al. 2020). In addition, Moro et al. (2019) indicated that chronic administration of NAC has long-lasting effects for up to 50 days post-treatment(Moro et al. 2019). Interestingly, Moro et al. (2019) observed that NAC administration during abstinence in the home cage failed to reduce cue-induced reinstatement, but administration during experimental cue-exposure therapy or during extinction sessions attenuated cue-induced seeking. This suggests pairing NAC treatment with experimental cue-exposure therapy or extinction sessions may increase the effectiveness of NAC to prevent relapse (Moro et al. 2019). These authors also reported that seven days post experimental cue-exposure therapy was associated with a lower expression of GLT-1 as well as higher expression of GluN2B in the AcbSh of nicotine self-administering rats, which was normalized by NAC treatment (Moro et al. 2019). Fifty days after NAC treatment there was a steep increase in mGluR2 levels in both the AcbSh and AcbCo, as well as normalization of xCT expression in the AcbCo, and normalization of GLT-1 expression in the AcbSh suggesting that NAC treatment can induce long-term increases in glutamate uptake (Moro et al. 2019).

Namba et al. (2019) found that NAC normalized GLT-1 expression in the AcbCo, reduced tumor necrosis factor-alpha (TNFα) expression in the AcbCo, and suppressed α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor to NMDA current ratios, which again suggests NAC acts to restore glutamate homeostasis and attenuate inflammatory response induced by cue-induced nicotine-seeking following nicotine self-administration. Bowers et al. (2016) indicated that NAC reduced the development of a nicotine conditioned place preference, nicotine somatic withdrawal signs, hyperalgesia, while inducing a conditioned place aversion in mice. However, it did not alter palatable food conditioned place preference, anxiety-like behavior, or motoric capacity. In alcohol-preferring UChB rats, oral administration of NAC reduced oral nicotine intake and fully suppressed the reinstatement of a nicotine conditioned place preference (Quintanilla et al. 2018). Moreover, NAC administration fully abolished increased oxidative stress and the neuroinflammatory markers induced by nicotine (Quintanilla et al. 2018). Clinical studies have shown that smokers treated with NAC reported a reduction in the number of cigarettes smoked (Knackstedt et al. 2009; McClure et al. 2015) and rated the first cigarette after an abstinence period as less rewarding (Schmaal et al. 2011). However, these effects were limited because NAC did not have any significant effects on craving (Knackstedt et al. 2009; Schmaal et al. 2011), withdrawal symptoms (Knackstedt et al. 2009; Schmaal et al. 2011), or breath carbon monoxide levels, which is a biomarker for smoking abstinence (Knackstedt et al. 2009). Furthermore, the majority of smokers did not maintain abstinence (Knackstedt et al. 2009; McClure et al. 2015). In contrast, a more recent study reported NAC treatment reduced craving, helped participants to maintain abstinence, and positively affected dysregulated corticostriatal connectivity (Froeliger et al. 2015). Thus, NAC

may act to alter reward processing thereby helping smokers to maintain abstinence immediately following cessation of smoking (Froeliger et al. 2015). Taken together, these findings suggest that NAC may have some efficacy in relapse prevention with regard to smoking.

There have been several clinical studies examining the efficacy of NAC in cocaine-using as well as -dependent subjects. In actively using cocaine-dependent individuals NAC did not alter cocaine use (LaRowe et al. 2013), however, there was evidence that it helped maintain abstinence in individuals who had already achieved abstinence (LaRowe et al. 2013). A more recent study found that cocaine use and problems (Drug Use Disorder Identification Test) were decreased with NAC treatment (Schulte et al. 2018). Lower cocaine-positive urine scores in the NAC group supported these findings (Schulte et al. 2018). Levi Bolin et al. (2017) indicated that NAC treatment significantly attenuated the reinforcing effects of cocaine. However, NAC has had mixed results on psychostimulant craving. It has been shown to reduce cocaine craving (Amen et al. 2011), although others did not find similar effects on craving or self-reported abstinence (Schulte et al. 2018). Also, NAC did not have an effect on cocaine cue-reactivity-associated neural correlates (Schulte et al. 2019). Nevertheless, others have found that NAC suppresses methamphetamine-craving (Mousavi et al. 2015). In early work, the administration of NAC, during extinction, inhibited cue-induced and heroin-primed reinstatement of heroin-seeking with long-lasting effects up to 40 days post-treatment (Zhou and Kalivas 2008). These findings suggest that repeated NAC administration may have therapeutic potential in enhancing abstinence and reducing drug-seeking behaviors and -craving.

14.15 Conclusions

SUDs are characterized by a long-lasting vulnerability to relapse across drug classes. Prolonged neuropathological changes to the glutamatergic system, within the MCL described above, appear to contribute to the addicted state through glutamate dysregulation. The significance of glutamate in learning and memory implicates the magnitude of its role in initiating and promoting addiction, Alzheimer's disease, posttraumatic stress disorder (PTSD), and other psychiatric conditions. The impact of glutamate transport and maintaining homeostasis to avoid neurotoxicity and damage from oxidative stress necessitates additional investigation of EAATs and vGluTs. Further research into the distinct neuroadaptations that result from glutamate dysregulation could provide information needed to develop more effective pharmacotherapeutics to treat addiction. Preclinical research has begun to explore the potential of glutamate transporters as therapeutic targets through NAC and cefazolin. Importantly, continued examination of the mechanisms behind the altered MCL and response to rewarding stimuli following chronic drug exposure may also support the development of pharmacotherapies for individuals with a dual-diagnosis of an SUD comorbid with another psychiatric disorder.

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Chapter 15
Glutamate in Multiple Sclerosis: From Pathophysiology to Treatments

Anna Pittaluga and Guendalina Olivero

Abstract Multiple sclerosis (MS) is an autoimmune disease typified by overt demyelination and inflammation that develop in selected regions of the central nervous system (CNS). Besides these signs, a diffuse loss of synaptic contacts, axonal pruning and astrocytosis are also observed, that in general correlate with the dysregulation of the glutamatergic system and with the onset of neurological symptoms. Concomitantly to the synaptic derangements, impaired glutamate homeostasis also dysregulates the immunocompetent responses, impairing the functional cross-talk between the immune system and the CNS. The study of the glutamatergic system therefore emerges as an important issue for deciphering the cellular events at the basis of MS as it would permit the proposal of new appropriate pharmacological interventions for the cure of the pathology. The chapter describes recent advances in basic research, preclinical and clinical studies concerning the impact of altered glutamate homeostasis in the course of the disease, as well as in the innovative strategies that would permit the restoration of central glutamatergic transmission.

Keywords Multiple sclerosis · EAE mice · Glutamate · Synaptopathy · Release · Uptake · Receptors

Abbreviations

2-AG	2-arachidonoylglycerol
2-PMPA	2-(phosphonomethyl) pentanedioic acid

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_15

AC	Adenylyl cyclase
CB1 receptors	Cannabinoid receptors type 1
CNS	Central nervous system
d.p.i.	Days post immunization
DMDs	Disease-modifying drugs
EAAT	Excitatory amino acid transporter
EAE	Experimental autoimmune encephalomyelitis
EC	Endogenous cannabinoids
EPSCs	Excitatory postsynaptic currents
FAAH	Fatty acid amide hydrolase
GCPII	Glutamate carboxypeptidase II
GDH	Glutamate dehydrogenase
GLS	Glutaminase
GOT	Glutamate-oxaloacetate transaminase
GPT	Glutamate-pyruvate transaminase
GS	Glutamine synthase
HCAR2	Hydroxycarboxylic acid receptor 2
IPSCs	Inhibitory postsynaptic currents
IS	Immune system
KA	Kynurenic acid
KP	Kynurenine pathway
LTD	Long-term depression
LTP	Long-term potentiation
MBP	Myelin basic protein
MGL	Monoacylglycerol lipase
mGlu receptor	Metabotropic glutamate receptor
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
MUNC-18	Mammalian uncoordinated-18
NAA	*N*-acetyl-aspartate
NAALADase	*N*-acetylated-alpha-linked acidic dipeptidase
PKA	Protein kinase A
PLP	Proteolipid protein
PPMS	Primary progressive multiple sclerosis
QA	Quinolinic acid
RMI	Resonance imaging
RRMS	Relapsing-remitting multiple sclerosis
SPMS	Secondary progressive multiple sclerosis
T	Tryptophan
TMS	Transcranial magnetic stimulation
xCT	Cystine/glutamate antiporter

15.1 Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) affecting about two to three million people worldwide. It is a progressively degenerating disorder typified by autoimmune attack directly at antigens associated with myelin, leading to the appearance of focal demyelinated plaques within the brain and the spinal cord. Despite the great efforts to investigate its aetiology and although much has been discovered about the immunobiology, genetics and epidemiology of the disease, its aetiopathogenesis remains so far unknown.

In most patients, MS develops a fluctuating course, typified by early relapsing-remitting episodes of neurological and radiological worsening, followed by symptomatologic recovers (i.e. the relapsing-remitting MS, RRMS). Epidemiological studies show that the 85% of patients suffering from MS develops the RRMS form of the disease. Within a decade, a large part of these individuals progresses to a secondary progressive MS (SPMS), that is typified by the development of neurological deficits that occur independently from relapses. A minor percentage (~15%) of patients develops a progressive course of the disease (i.e. the primary progressive MS, PPMS) for unknown reasons (Lublin et al. 2014).

Autoimmune mechanisms brought about by inflammatory lymphocytes, macrophages and activated microglia are traditionally proposed to play the major role in the development of the pathology. In particular, it is proposed that MS is initiated and maintained by the continuous migration of inflammatory immune cells from the periphery into the CNS, but also by the concomitant modulation of the autoimmune attack, probably mediated by the infiltrating T regulatory cells themselves. Recruitment of pro-inflammatory and regulatory leucocytes into inflamed tissues is controlled by chemokines and their receptors through their ability to drive gradient-dependent cell migration nearby the sites where they are actively released (Karpus and Ransohoff 1998; Ransohoff et al. 2007). Accordingly, the increased expression of selected chemokines (CCL5 and CXCL12, for instance) are predictive markers of the progression of the disease (Besong et al. 2002; Godiska et al. 1995; Pittaluga 2016; Sørensen et al. 1999).

Despite the peripheral to central immunological events are thought to play a main role in the development of the disease, they are not essential to the onset of central derangements. In fact, synaptic impairments, grey matter lesions and demyelination become evident in selected CNS areas (i.e. the cortex, the hippocampus, the cerebral cortex, the thalamus and the caudate-putamen) of MS patients starting from the earliest phases of the disease, also in the absence of infiltrating lymphocytes and macrophages (Klaver et al. 2013, Bevan et al. 2018; Eshaghi et al. 2018). The brain magnetic resonance imaging (RMI) is usually used to evidentiate these central lesions, that if present, often correlate with the onset of the early clinical neurological symptoms of the disease, consistent with their relevance in the course of the pathology.

Based on these considerations, starting from the last decade, MS has been classified also as a primary neurodegenerative disorder and two terms were proposed

to describe its course. The first term is "synaptopathy", to evidentiate the main role of synaptic disruption in the pathological framework (Mandolesi et al. 2015a, b) and the second one is "silent progression", which refers to the mode of progression of the disease, to stress the fact that neurodegeneration proceeds in MS patients largely independently from autoimmune inflammation (17; University of California, San Francisco MS-EPIC Team, and Cree 2019).

Our understanding of MS, as well as of the development of disease-modifying therapies, mostly relies on the availability of disease animal models. Among the available models, most of the preclinical data originate from studies carried out in mice suffering from the experimental autoimmune encephalomyelitis (the EAE mice). The EAE mice recapitulate many features of MS (Rangachari and Kuchroo 2013; Swanborg 1995). The demyelinating disorder is induced by immunizing mice with myelin antigens including myelin basic protein (MBP), proteolipid protein (PLP) or the myelin oligodendrocyte glycoprotein (MOG). In particular, the immunization with MOG elicits the development of a non-remitting form of disease typified by the presence of inflammatory lesions and demyelinated areas that predominate in the spinal cord. The clinical signs become evident at the early almost asymptomatic stage of the disease (at about 13 days after immunization, d.p.i.) and reach the maximal gravity at about 21 $\pm$ 1 d.p.i.

Interestingly, beside central inflammation and demyelination, EAE mice also develop altered glutamatergic transmission in selected CNS regions (i.e. the cortex), independently on the presence of clear white matter injures (Mangiardi et al. 2011). The EAE mice therefore represent a suitable model to study the neurological defects and the altered synaptic plasticity that typify the course of the disease.

15.2 The Glutamatergic System in MS and in EAE Mice

Glutamate controls the homeostasis of the CNS. It determines synaptic plasticity, i.e. the principal neuronal property involved in the ability of CNS to resist to insults, to assure an efficient neuronal response to stimuli and to build up restorative adaptations. In other terms, glutamate participates to determine the cognitive reserve, i.e. the ability of the brain to recover the maladaptation elicited by injures and/or aging during the entire lifespan. These beneficial effects however can be exhausted when pathological conditions that alter bioavailability of glutamate persist during time and/or overwhelm the mechanisms of "cognitive buffering".

In the CNS, the majority of neurons are glutamatergic and glutamate represents the principal transmitter at chemical synapsis. Derangements of glutamatergic homeostasis have a detrimental impact on the CNS and prelude to neurotoxicity as well as to synaptic dysregulation. In MS patients impaired glutamatergic (and GABAergic) synaptic transmission can be evidentiated by using the transcranial magnetic stimulation technique (TMS, Stampanoni Bassi et al. 2017). This synaptic maladaptation is proposed to play a main role in the onset and the development of the central lesions and neurological disability that typify the course of the disease. In

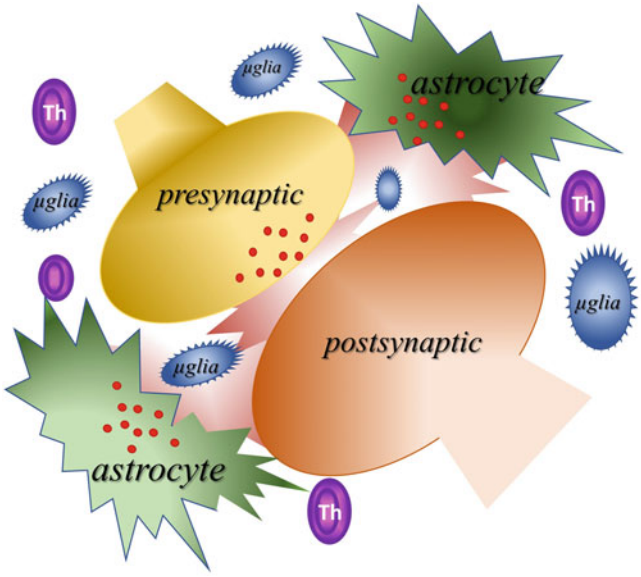

Fig. 15.1 Glutamate is the main excitatory transmitter in the central nervous system and plays a main role in controlling synaptic plasticity, to assure an efficient neuronal response to stimuli and to build up restorative adaptations. Unfortunately, when pathological conditions prevail and bioavailability of glutamate itself persists and overwhelms the mechanisms of "synaptic buffering", glutamate becomes detrimental, favouring the mechanisms of neurodegeneration and altering the efficiency of synaptic connection. In the central nervous system, besides neurons, which represent the primary physiological source of the excitatory amino acid, there are several other cells [i.e. astrocytes, microglia (μglia), brain macrophages, dendritic cells and infiltrating leucocytes and lymphocytes (Th)] that release glutamate and that control its homeostasis. These cells, in particular astrocytes and migrating lymphocytes, are important sources of the excitatory amino acid in pathological conditions associated with inflammation or immunocompetent responses, such as those observed in MS

humans, learning and memory as well as the resilience to stress and anxiety largely depend on the efficiency of central glutamate transmission and it is recognized that the deterioration of the glutamatergic innervation negatively reverberates on these functions. Consistent with this view, the neurological symptoms often observed in MS patients include difficulties in learning and remembering new information, depression and anxiety. These symptoms are observed in approximately 50% of individuals with MS and, unfortunately, in some cases (at least in early MS) are neglected or misdiagnosed (for reviews, see (Passos et al. 2014; Rao et al. 1991; Siegert and Abernethy 2005)).

Besides neurons, which represent the primary physiological source of the excitatory amino acid, several other cells (i.e. astrocytes, glial cells, brain macrophages, dendritic cells and infiltrating leucocytes and lymphocytes) release glutamate and control its homeostasis. Astrocytes and migrating lymphocytes are important sources of glutamate in pathological conditions associated with inflammation or

immunocompetent responses, such as those observed in MS (Fig. 15.1). All these aspects have been largely discussed in previous reviews and will be not further analysed in this chapter (see for exhaustive review (Centonze et al. 2009; Di Filippo et al. 2015; Fazio et al. 2018; Levite 2017; Mandolesi et al. 2015b; Matute et al. 1999; Nicoletti et al. 2011; Pittaluga 2017)).

15.2.1 Glutamate Bioavailability in the CSN of MS Patients and EAE Mice

Glutamate represents the driving force for synaptic plasticity and, as already introduced, observations in MS patients and EAE animals agree with the conclusion that severe, region-dependent alterations of glutamatergic transmission in the CNS are pivotal to disease.

In 1997, Klivényi and colleagues (Klivényi et al. 1997) quantified the levels of amino acids in the CSF of MS patients and compared the results with those obtained from the CSF of individuals suffering from lower back pain. The authors did not find significant differences in the CSF concentrations of the amino acids between the two groups. Almost concomitantly Stover et al. (1997) found that the level of different amino acids (including glutamate) was almost doubled in the CSF of MS patients at the acute symptomatic phase of the disease with respect to healthy subjects. Sarchielli et al. (2003) demonstrated a significant increase of glutamate (and aspartate) levels in the cerebrospinal fluid (CSF) of patients with the RRMS and the SPMS when compared to control individuals (subjects without central or peripheral neuronal pathology). They also observed a correlation between the phase of relapse and the concentration of glutamate in the CSF. Furthermore, they showed that the levels of glutamate in patients suffering from the RRMS but who had active lesions were higher with respect to patients without neuroradiological signs. High levels of glutamate were also detected in the CSF of patients suffering from SPMS.

In 2014 evidence was provided showing decreased level of glutamate in large areas of normal-appearing white and grey matter in MS patients (Azevedo et al. 2014). Multivoxel spectroscopy was used to quantify the glutamate and the *N*-acetylaspartate (NAA) levels and to quantify the GLU/NAA ratio in these patients. The results of the study unveiled a high GLU/NAA ratio that was considered predictive of altered neuroaxonal integrity. The result was proposed to be predictive of a decline of the brain volume and therefore of disease progression.

As to the EAE mice, data exists in the literature showing correlations between altered glutamate homeostasis and oligodendrocyte and axonal damage (Matute et al. 1999, 2001; Werner et al. 2001), among hyperglutamatergicity, neuroinflammation and synaptic degeneration (Mandolesi et al. 2010), as well as between altered glutamate release and glutamate receptor/transporters dysfunctions (as discussed below, but see (Castegna et al. 2011; Levite 2017; Pittaluga 2017)).

The excess of glutamate in the synaptic cleft is neurotoxic since it assures a pathological activation of the receptors repertoire (in particular the ionotropic glutamate receptors, namely NMDA and AMPA receptors) that is maladaptive to the synaptic network. The pathologically-relevant, increased availability of the amino acid might depend on several cascades of events, involving the impaired expression/functions of glutamate metabolizing enzymes, the dysfunction of the glutamate transporters and the hypersecretion of glutamate due to maladaptation in the synaptic machinery accounting for vesicular exocytosis, as well as to the overproduction of release-regulating factors, including cytokines. The information concerning these aspects is reviewed below.

15.2.2 Glutamate Metabolizing Enzymes in the CSN of MS Patients and EAE Mice

Studies in the literature correlate the central altered glutamate availability with the impaired expression/functions of glutamate metabolizing enzymes [i.e. the glutamate dehydrogenase (GDH) and the glutamine synthase (GS)] as well as of enzymes which tune the production of the amino acid [i.e. the glutaminase (GLS)]. As the GLS is concerned, its overexpression was reported to correlate with axonal damage. In particular, both early and chronic active lesions in brain tissue from MS patients showed high levels of the enzyme. Differently, evident GLS immunoreactivity was not observed in chronic silent lesions. The GLS-positive cells mirrored the distribution of activated macrophages and microglia cells, which are characteristic of central inflammation, suggesting a cross-linking between the overexpression of GLS-containing glutamate-producing immune cells and the development of excitotoxicity in the CNS of MS patients (Werner et al. 2001).

GS and GDH expression was dramatically decreased in the spinal cord of EAE mice with a very high score (Hardin-Pouzet et al. 1997). Increased oxidative modifications of GS in the cortex of EAE mice paralleled the severity of the clinical signs, while the GS/glutamate ratio largely decreased suggesting a correlation between EAE severity and excitotoxicity (Castegna et al. 2011). In the CNS of MS patients, the distribution of GDH and GS immunoreactivity was largely different from non-MS tissues. In particular, both enzymes were poorly expressed in oligodendrocytes but largely present in astrocytes and microglia (Werner et al. 2001).

15.2.3 Efficiency of Central Glutamatergic Transmission in the CNS of MS Patients and EAE Mice

The efficiency of synaptic transmission in MS patients is usually analysed with non-invasive techniques including the Transcranial Magnetic Stimulation (TMS) to

assess the integrity of the motor cortex plasticity, the efficiency of the cortical-spinal innervation and the efficiency of local interneurons, i.e. the GABAergic inhibitory ones for instance, in modulating synaptic signalling. Specific TMS measures and different protocols of stimulations (exhaustively described by (Stampanoni Bassi et al. 2017)) can be applied to evaluate the efficiency of glutamatergic transmission. The revision of the data in the literature suggests that the observations so far available require careful evaluation because of the heterogeneity of the modalities adopted for the recruitment of both the healthy and the MS patients, of the form of the disease they suffer from and of the concomitance of on-going therapy. Nonetheless, the results permit the conclusion that brain networks that are relevant to vision, cognition or sensory-motor functions undergo progressive modifications in the excitatory transmission (and in the GABAergic one as well) starting from the onset of the demyelinating disorder and that these modifications can be maladaptive in nature. These neuronal alterations do not cause irreversible modification in the synaptic activity, at least at the early stages of the disorder. As a matter of fact, the available results seem compatible with the conclusion that, at the onset of the disease, the brain maintains the ability to cope with the local and diffuse neuronal damages, resisting until its resilience to injures is not exhausted by the maladaptive stimuli. This synaptic flexibility accounts for the discrepancy often observed between the clinical disability and the central lesions in patients, suggesting that the ability of CNS to compensate for the central injures has an efficacy and an intensity that vary among individuals (Di Filippo et al. 2013, 2015; Weiss et al. 2014).

Synaptic plasticity, as well as glutamatergic and GABAergic transmission, was also analysed in the available animal models of demyelinating disorders, in particular in the EAE mice, by using different approaches typified by a different level of anatomical and functional complexity.

Synaptic plasticity originates from the mechanisms of the Long-Term Potentiation (LTP) and of the Long-Term Depression (LTD, Malenka and Bear 2004). LTP is the persistent increase in efficiency of transmission at excitatory synapsis (Bliss and Lomo 1973) while LTD consists of a decreased synaptic transmission that it is produced by prolonged low-frequency stimulation (Mulkey and Malenka 1992). LTP depends on glutamatergic signalling mainly involving NMDA receptors and can be manipulated pharmacologically either by controlling glutamate signalling or by modifying the GABAergic innervation. LTD involves glutamatergic signalling as well, but it is also mediated by metabotropic glutamate receptors (Jones 2017). Interventions that could affect the efficiency of glutamatergic and GABAergic transmissions permit to manage and promote the synaptic plasticity and its functional reorganization, increasing therefore the resilience of CNS to the neuronal injuries that develop in the course of several central disorders including MS.

As far as the synaptic plasticity in EAE animals is concerned, studies dedicated to quantify the efficiency of LTP and LTD in the hippocampus (Di Filippo et al. 2013; Mori et al. 2014; Mosayebi et al. 2016; Nisticò et al. 2013; Novkovic et al. 2015; Prochnow et al. 2013; Weiss et al. 2014) permitted to explore the gravity of synaptic impairments that occur during the course of the demyelinating disorder. Studies were

mainly carried out in the hippocampus of EAE mice at the acute stage of disease or soon after, at the chronic phase. The available data agree upon the reduction of LTP efficiency when compared to healthy controls, an event that strictly correlates with the gravity and the progression of the symptoms in the EAE animals. These maladaptive events are expected to play a main role in determining a progressive exhaustion of the central neuronal plastic reserve and indirectly accounts for the development of clinical signs such as cognitive defects, impaired locomotor activity, mood and social impairments that emerge when testing behavioural skills in EAE mice (Acharjee et al. 2013; Di Prisco et al. 2014a; Olechowski et al. 2013).

Beside these studies, electrophysiological recordings in slices were also carried out to evaluate specifically the efficiency of glutamate and GABA transmission in selected regions of the CNS. The studies focussed on the analysis of the excitatory (EPSCs) and the inhibitory postsynaptic currents (IPSCs) at chemical synapses. The pre- and the postsynaptic excitatory signalling was found to be increased in cortico-striatal slices from EAE mice at the early and the acute stage of disease (Grasselli et al. 2013; Haji et al. 2012; Rossi et al. 2010, 2011, 2012) and in the basolateral amygdala (Acharjee et al. 2018), but it was significantly decreased in hippocampal slices of EAE mice at the acute and the chronic stage of disorder progression (Di Filippo et al. 2013; Mosayebi et al. 2016; Ziehn et al. 2012), further suggesting the region-specificity of the synaptic impairments subserving the electrophysiological recordings. Interestingly, in the striatum of EAE at the symptomatic phase the increased EPSP signalling was paralleled by a significant reduction of the IPSP signalling (Mandolesi et al. 2013; Musumeci et al. 2011) that would amplify the altered EPSPs signalling observed in this brain region.

Finally, a direct quantification of the amount of glutamate and GABA release in nerve terminals was achieved by using purified synaptosomes. Interestingly, the dissection of the neuronal component from slices to synaptosomes unveiled a scenario even more complicated, depending on the CNS regions under study and on stage of the disease. The efficiency in glutamate exocytosis was found to be significantly increased in spinal cord synaptosomes of symptomatic EAE mice during and after the acute stage of disease (Bonfiglio et al. 2017; Di Prisco et al. 2013, 2014a, b, 2016; Marte et al. 2010), which would be consistent with the increased glutamate availability observed in the CSF of MS patients and EAE mice as well. In this region, also the GABA exocytosis was potentiated, consistent with the conclusion that the EAE-induced deregulation of exocytosis is a general event that relies on functional modifications of the intraterminal machinery subserving the recruitment of the release vesicles, independently on the neuronal population and on the neurotransmitter actively released. According to this view, it was shown that the cytosolic adenylyl cyclase (AC) dependent, protein kinase A (PKA)-mediated events, as well as the endogenous production of inositol triphosphate in spinal cord synaptosomes from EAE mice at the acute stage of disease, are largely increased when compared to healthy animals (Di Prisco et al. 2013). Considering that both events participate to determine the cytosolic calcium availability in neurons, it seems conceivable to propose that these metabolic adaptations might be

critical to determine the increased release efficiency detected at both spinal cord glutamatergic and GABAergic synaptic boutons.

Opposite to the spinal cord, the efficiency of glutamate exocytosis was drastically reduced in cortical synaptosomes. The negative adaptation became evident very early, when the animals became symptomatic (i.e. 13 ± 1 d.p.i.), and persisted during and after the acute stage of disease. Also in this case the onset of glutamate release defects was paralleled by significative changes in AC e PKA activities, that were drastically reduced in this CNS region (Chanaday et al. 2015; Cid et al. 2011; Di Prisco et al. 2013; Vilcaes et al. 2009). Finally, impaired glutamate exocytosis emerged in hippocampal synaptosomes only after the acute stage of disease (35 ± 1 d.p.i., (Bonfiglio et al. 2017)). Evident changes in GABA exocytosis were not observed at all the stages of the disease in both cortical and hippocampal synaptosomes (Bonfiglio et al. 2017; Di Prisco et al. 2013, 2014a, b, 2016).

Studies were also dedicated to investigate whether changes in glutamate exocytosis efficiency observed in EAE mice were paralleled by modifications of the expression of proteins involved in the mobilization of transmitter vesicles at presynaptic nerve endings. It was found that the reduced exocytosis of glutamate detected in EAE animals coincides with alterations of the presynaptic machinery. In particular, the kinetic of the calcium-dependent phosphorylation of synapsin I was significantly decreased in cortical synaptosomes of EAE rats (Chanaday et al. 2015; Vilcaes et al. 2009), an event well consistent with the reduced mobility of the synaptic vesicles. Furthermore, the expression of the mammalian uncoordinated-18 (MUNC-18) protein was reduced in cortical nerve endings from EAE mice, suggesting the destabilization of the synaptic vesicle fusion complex (Bonfiglio et al. 2019). Finally, the expression of synapsin-2 and synaptotagmin-1 in the serum of EAE mice was modified (Raphael et al. 2017).

15.2.4 Glutamate Transporters Expression and Function in the CSN of MS Patients and EAE Mice

Altered glutamate availability in the synaptic cleft depends on impaired exocytosis but also on the impaired mechanisms of reuptake in astrocytes, neurons, oligodendrocytes and immune-competent cells (Fig. 15.2). In physiological conditions, the transporters expressed in all these cells rapidly remove, although to a different extent and with different efficacy, the glutamate in the synaptic cleft, strictly controlling the efficiency of the excitatory postsynaptic signalling which correlates with the concentration of the excitatory amino acid in the biophase. The main players in these cellular events are the excitatory amino acid transporters (EAATs, Danbolt et al. 2016) and the cystine/glutamate antiporter (xCT). As far as the EAATs are concerned, to date five members of the family have been described, which predominate in the CNS and that are typified by a preferential anatomical distribution. The EAAT1 transporter has a main non-neuronal expression, being preferentially

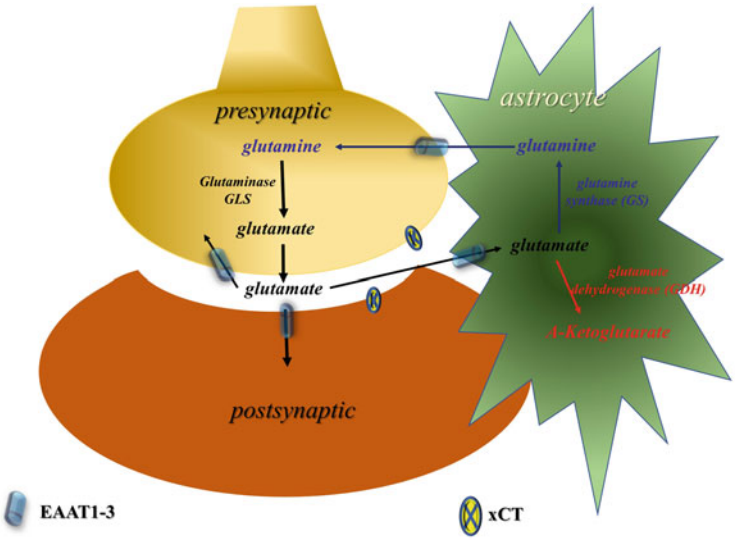

Fig. 15.2 Representation of glutamate transport and metabolism at the tripartite synapsis. Glutamate released by nerve endings and astrocytes can diffuse in the synaptic biophase but it is also actively taken up by astroglial and neuronal excitatory amino acid transporters (EAAT 1–3), as well as by cystine/glutamate antiporter (xCT) that indirectly dictate the strength and the efficiency of glutamate signalling. Glutamate taken up by astrocytes is the substrate of glutamine synthase (GS) to produce glutamine that diffuses to the presynaptic component of glutamatergic synapses to produce glutamate by means of glutaminase (GLS). Glutamate in astrocytes is also substrate of glutamate dehydrogenase (GDH) to produce α-ketoglutarate. The availability of glutamate at synapsis is therefore critically dependent upon diffusion, metabolism and active uptake through selected glutamate transporters, representing therefore a complex system that can be targeted at different levels by pathological conditions typified by inflammation and autoimmune responses

expressed in subpopulation of glial cells, i.e. the Bergmann glia and the Muller cells, while the EEAT2 locates in astrocytes and accounts for most of the glutamate uptake. Differently, the EAAT3, 4 and 5 subtypes are mainly expressed in neurons, the EEAT4 in cerebellar Purkinje cells and the EEAT5 in the retina (Dunlop 2006).

EAAT subtypes impact differently the course of central neurological diseases, including MS or EAE, and their activity depends on the phase of the pathology. In general, at the acute symptomatic stage of EAE, EAAT1 was reported to undergo adaptive modifications preferentially leading to the reduction of mRNA and protein expression, that would be consistent with a reduced efficacy of the mechanism of synaptic protection. Differently, contradictory results are available on the impact of the demyelinating disorders on EAAT2 and the EAAT3 proteins, that were reported to be either increased, decreased or unaffected during the course of disease (Azami Tameh et al. 2013; Mandolesi et al. 2015a, b; Melzer et al. 2008; Mitosek-Szewczyk et al. 2008; Ohgoh et al. 2002; Sulkowski et al. 2009; Vallejo-Illarramendi et al. 2006; Werner et al. 2001). Interestingly, by a functional point of view, glutamate was taken up more efficiently in both nerve endings (synaptosomes) and glial

particles (gliosomes) purified by the spinal cord of EAE mice at the early asymptomatic stage of disease (Marte et al. 2010).

Last but not least, in 2008 the group of Domercq reported the presence of a polymorphism in the promoter of the EAAT2 protein leading to a significant reduction of the expression of the transporter expression. The polymorphism is not associated with an increased risk to develop MS, but it is associated with high glutamate plasma levels during the course of a relapse in RRMS patients (Pampliega et al. 2008).

Besides EAATs, the xCT antiporter also influences glutamate bioavailability in the synaptic cleft. The xCT is a membrane transport system that assures the uptake of extracellular cystine (i.e. the limiting factor in the biosynthesis of glutathione, which has a key role in antioxidant defence) and the concomitant outflow of glutamate in most cells, including oligodendrocytes. xCT consists of two subunits, namely xCT and 4F2hc that heterodimerize. The xCT light chain determines the specificity of the amino acid transport, whereas the 4F2hc protein is common to several amino acid transporters and assures the correct insertion of the antiporter in membrane. Because of the main role of the two substrates in controlling the brain functions, functional maladaptation of this antiporter could be detrimental to central homeostasis and in particular to the myelinated fibres (Soria et al. 2016). In particular, upregulation of the xCT is protective to oxidative stress since indirectly potentiates the intracellular biosynthesis of glutathione to improve reactive oxygen species detoxification. The dysregulation of the xCT causes increased release of glutamate in the biophase, participating to glutamate-mediated excitotoxicity. In 2011 Pampliega and colleagues (Pampliega et al. 2011) demonstrated that the xCT light chain is overexpressed in the CNS and in peripheral blood cells (i.e. cells from monocyte-macrophage-microglia lineage) in MS patients as well as in EAE mice. These findings allowed the conclusion that upregulation of xCT antiporters represents a maladaptive event of the demyelinating disorder that may favour overt hyperglutamatergicity and excitotoxic damage to oligodendrocytes.

In a whole, the observations so far discussed agree with the conclusion that the functionalities of most of the regulatory systems that control the glutamatergic innervation are selectively altered during MS and or EAE and cannot correctly assure the excitatory transmission required to maintain synaptic plasticity.

15.2.5 Glutamate as Modulator of the Immune System: Central Nervous System Cross-Talk

Although the CNS has been long considered an immune-privileged organ, it is now widely recognized that the immune system (IS) plays a main role in the development of central neurodegenerative diseases (i.e. Alzheimer's disease or amyotrophic lateral sclerosis) as well as in classic autoimmune-inflammatory disorders (MS). In support to this conclusion evidence suggests that many endogenous

immunocompetent molecules (i.e. cytokines and chemokines) are produced and released in the CNS where they control synaptic transmission, being therefore specific signalling molecules linking the IS and the CNS (Besong et al. 2002; Centonze et al. 2009; Rostène et al. 2007).

Central resident immunocompetent cells and/or peripheral T cells migrating to the CNS can efficiently release glutamate. Furthermore, these cells also express glutamate receptors sensing therefore the changes in glutamate homeostasis in the brain. Finally, myelin-reactive T cells provoke microglia to release glutamate through the system xCT transporter promoting myelin degradation in EAE (Evonuk et al. 2020). All these aspects have been deeply revised by other authors (Levite 2017) and will not be further discussed in this chapter.

15.3 Glutamate Receptors as Potential Drug Targets for Treating Autoimmune Demyelinating Disease

Glutamate exerts its action at chemical synapses by binding glutamatergic receptors that locate synaptically, both at the pre- and at the postsynaptic components of the synapsis, as well as in surrounding cells, such as astrocytes and microglia. Glutamate receptors also exist in oligodendrocytes and oligodendrocyte progenitor cells as well as in immunocompetent cells, where they drive the myelination and the immunocompetent activities of the CNS.

Glutamate receptors consist of ionotropic and metabotropic receptors which have been proposed to participate to a different level and with different impact to the onset and the development of MS (Fig. 15.3).

15.3.1 Ionotropic Glutamate Receptors in the CSN of MS Patients and EAE Mice

In 2000, two different laboratories provided evidence showing that the treatment with an AMPA antagonist (i.e. NBQX) largely reduced the neurological deficits in EAE mice causing a substantial amelioration of the clinical scores, increasing oligodendrocytes survival and reducing axonal lesions (Pitt et al. 2000; Smith et al. 2000). Both the groups proposed that the AMPA-induced beneficial effects did not rely on anti-inflammatory activity, since NBQX-treatment had no effect on lesion size and did not reduce the degree of central inflammation. In addition, NBQX did not alter the proliferative activity of antigen-primed T cells in vitro, suggesting that an immunomodulatory activity was not primarily involved. Rather, it was proposed that AMPA receptors mediate the neurological sequelae of events that sustain the disease progression and therefore that their blockade could be beneficial to the course of the pathology (Pitt et al. 2000; Smith et al. 2000; Werner et al. 2000).

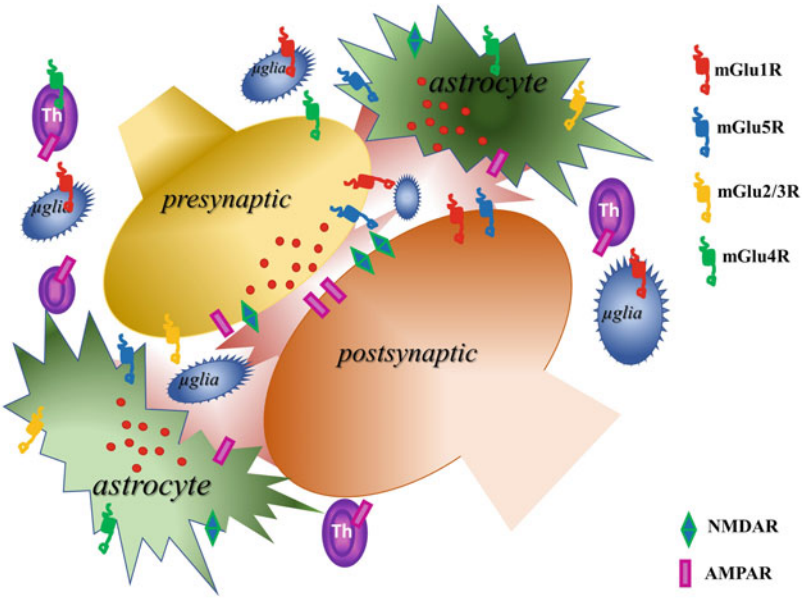

Fig. 15.3 Glutamate exerts its action by binding glutamatergic receptors that locate synaptically, both at the pre- and at the postsynaptic components of the synapsis, as well as in surrounding cells, such as astrocytes and microglia (μglia). Glutamate receptors also exist in oligodendrocytes and oligodendrocyte progenitor cells, as well as in immunocompetent cells, where they drive the myelination and the immunocompetent activities of the CNS. Glutamate receptors consist of ionotropic and metabotropic receptors which have been proposed to participate to a different level and with different impact to the onset and the development of MS. Drugs that influence the expression and the functions of glutamate receptors could permit to partly recover the central derangements and pathological signalling underlying the onset and the progression of demyelinating disorders

The role of AMPA receptors in dictating the recruitment and the migration of T cells in the CNS, however, was soon after demonstrated by Ganor et al. (Ganor et al. 2003), which provided convincing evidence that normal human T cells, human T leukaemia cell, and mouse anti-myelin basic protein T cells express high levels of GluA3-containing AMPA receptors, the activation of which drives the CXCR4-mediated T cell chemotactic migration towards the site of release of the chemokine CXCL12 in the central inflammation loci. The role of AMPA receptors in determining the progress of the disease was confirmed by Kanwar et al. (2004). In particular evidence was provided showing that GluA3 subunits are pivotal in the development of neuronal deficits in EAE mice. In fact, mice genetically modified for the expression of GluA3 subunits were more resistant to neuronal excitotoxicity and developed a milder spinal demyelination when immunized with myelin oligodendrocytes 35-55 protein (MOG35-55, Bannerman et al. 2007). Almost concomitantly, Sarchielli et al. (2007) provided evidence showing that T lymphocytes of control subjects and MS patients express both mRNA and protein of GluR3 receptors and that the activation of the GluA3-containing AMPA receptors enhances the proliferation and the

chemotactic migration of T lymphocytes from both controls and MS patients (but see also Dutta et al. (2013); Newcombe et al. (2008)).

An interesting aspect refers to the ability of AMPA receptors to undergo constitutive trafficking in neuronal plasma membranes, an event that controls the insertion and the efficiency of the AMPA receptor-mediated signalling (Henley 2003; Pittaluga et al. 2006). AMPA receptor trafficking is controlled by several agents, including the immediate early gene Arc/Arg3.1, that has a preferential postsynaptic localization and that regulates the AMPA receptor insertion in neuronal plasma membranes, prolonging LTP (Chowdhury et al. 2006). In mice with EAE between 20 and 30 d.p.i., Arc/Arg3.1 mRNA level was dramatically downregulated in the striatum. This event was proposed to participate in the reduction observed in this region of the strength of AMPA-mediated excitatory transmission and, taking into account that the Th1 cytokines (IFNg, TNFa and IL-1b) can modulate the Arc/Arg3.1 mRNA expression on primary neuronal cultures, it seems conceivable to propose a strong link between long-lasting synaptic changes and inflammation (Centonze et al. 2009).

As far as the NMDA receptors are concerned, the observations that LTP and LTD are impaired in EAE mice indirectly suggest the alteration in the expression/functioning of these receptors during the course of the disease (Di Filippo et al. 2013; Grasselli et al. 2013; Nisticò et al. 2013). The hypothesis is well in line with the results obtained in EAE mice administered with NMDA receptor antagonists. Treatment of EAE-sensitized animals with dizocilpine reduced the disease-associated increase in CNS levels of putrescine which is an endogenous negative modulator of the polyamine site at the NMDA receptors (reviewed by Bolton and Paul (2006)). Similarly, limiting NMDA receptor functions by administering EAE mice with memantine confirmed that pharmacological modulation of receptor function during EAE results in disease suppression and restoration of neurovascular function (Wallström et al. 1996).

In 2017, Lim and colleagues (Lim et al. 2017) investigated the metabolomic profile of the kynurenine pathway (KP) in MS patients. The KP is the major route of metabolism of tryptophan (T) and leads to the production of two main compounds, the quinolinic acid (QA) and the kynurenic acid (KA) which have opposite impacts on NMDA receptors. In particular, QA is an orthosteric agonist at NMDA receptor while KA is an antagonist that limits the NMDA-mediated signalling. In physiological condition, the QA/KA balance assures a correct activation of the NMDA receptors that supports the synaptic events. The activity of the pathway however is under the direct control of inflammatory agents. In particular, pro-inflammatory cytokines can elicit a dysregulation in the metabolic pathway, leading to an altered QA/KA ratio that may either favour excitotoxicity or impair the mechanisms of resilience and synaptic plasticity. QA is produced by activated microglia and infiltrating macrophages, but not by neurons or astrocytes, while KA is produced by astrocytes. The study involved two cohorts of patients suffering from the RR-SM, the SPMS and the PPMS; patients were analysed for the serum content of KA and T and identified for the KA/T ratio. In all the MS subtypes groups the K/T ratio was significantly increased compared to the healthy controls. Inasmuch, aberrant levels

of KA and QA were detected depending on the stage and on the form of the disease, leading to propose the KP metabolic signatures in patients as a marker with high sensitivity and specificity to discriminate clinical MS subtypes.

15.3.2 Metabotropic Glutamate Receptors in the CSN of MS Patients and EAE Mice

The metabotropic glutamate receptors consist of eight different receptor subtypes (namely, mGlu1 to mGlu8 receptor) that are further subdivided in main groups (group I, group II and Group III) based on the sequence homology, the coupled G protein and the associated transducing pathway(s) (Nicoletti et al. 2011; Pin and Acher 2002).

mGlu receptors are fundamental to the mechanism of synaptic plasticity and to the IS-CNS interactions, since they act as fine tuners of the functional responses of neurons and astrocytes as well as of the activation of microglia and immune-competent cells (Fazio et al. 2018).

The role of mGlu receptors in controlling chemical transmission and inflammation has been largely revised and will not be further addressed in this chapter (please refer to (D'Antoni et al. 2008; Fazio et al. 2018; Olivero et al. 2019; Pittaluga 2016; Raiteri 2008; Spampinato et al. 2018)).

Starting from the 2003, Aronica and colleagues provided evidence of changes in the expression of mGlu receptors belonging to the three groups in the CNS of MS patients. In particular, in 2003 (Geurts et al. 2003) they demonstrated that the expression of both group I and II mGlu receptors in MS tissues differed significantly from that of healthy individuals. Strong mGlu1a receptor immunoreactivity was observed in the subcortical white matter, particularly in the center of actively demyelinating lesions and in the borders of chronic active lesions. A diffuse increase in the expression of mGlu5 and mGlu2/3 receptors, but not of mGlu1a receptor, was also highlighted in reactive astrocytes, as well as in a population of microglial cells that displayed a macrophage-like morphology. Two years later, Aronica and colleagues (Geurts et al. 2005) also add insights concerning the group III. mGlu8 receptor immunoreactivity was detected in microglia/macrophage cells in the active lesions, but the expression in these cells significantly decreased in chronic active and inactive lesions. No mGlu4 receptors were detected in these lesions, but mGlu4 receptor immunopositivity emerged in a population of reactive astrocytes localized in the rim of the chronic lesions. More recently, in 2008, Fazio and colleagues demonstrated that, differently from what observed in other brain regions, the expression of the mGlu1a receptor is largely reduced in the Purkinje cells in the cerebellum of MS patients and this is paralleled by an increased expression of the mGlu5 receptors. In particular, the strong mGlu1a receptor somato-dendritic immunoreactivity in Purkinje cells of control human cerebellum was drastically reduced in the Purkinje cell/molecular cell layer of MS patients, while mGlu5 receptor

immunoreactivity, that it is not detectable in the Purkinje cell/molecular cell layer of healthy individuals, became prominent in Purkinje cells of the MS patients.

These observations in autoptic tissues from MS patients were largely replicated in the EAE mice (Besong et al. 2002). The expression of mGlu4 receptors in astroglial cells from EAE rats was significantly modified. To note, the activation of these receptors significantly reduced the production and release of the pro-inflammatory chemokine CCL5 which represent a marker of MS progression (Besong et al. 2002). Starting from 2010, however, evidence underlined the relevance of the mGlu4 receptor subtypes as potential target of therapy for the MS. Fallarino and colleagues (Fallarino et al. 2010) demonstrated that the genetic deletion of the mGlu4 receptors increases the susceptibility of mice to develop EAE, compatible with the conclusion that the overt hyperglutamatergicity observed during the progression of the disease might reflect a counter-regulatory mechanism that is protective in nature and that, by acting at mGlu4 receptors, it would provide a mechanism of defence in the progression of the pathology. Accordingly, it was demonstrated that the administration of cinnabarinic acid, an endogenous metabolite of the kynurenine pathway that acts as an orthosteric agonist of mGlu receptors, was highly protective against the development of EAE in mice (Fazio et al. 2014; Spampinato et al. 2015). Finally, besides mGlu4, also mGlu2/3 receptors were proposed to play a main role in the development of EAE signs in particular at the spinal cord level (Di Prisco et al. 2016). Group II mGlu receptors are known to have a preferential presynaptic localization in the central system and to control glutamate transmission at this level (Olivero et al. 2019). In symptomatic EAE mice, the release-regulating activity of the mGlu2/3 autoreceptors in the cortex was found to be largely reduced, but it was amplified in the spinal cord, suggesting these receptors as potential targets of new therapeutic approaches for controlling glutamate excitotoxicity in these regions.

15.4 Modulators of Glutamate Receptors for the Therapy of Autoimmune Demyelinating Disease

The relevance of the central glutamatergic transmission in the onset and the development of MS and demyelinating disorders is supported by the finding that several disease-modifying drugs (DMDs) currently in use for the cure of MS recover, at least in part, the central glutamate alterations in EAE mice.

Impaired glutamate release efficiency (measured as amount of transmitter release upon application of a depolarizing stimulus at the presynaptic level, as well as EPSPs frequency/intensity and as AMPA/NMDA ratio at the postsynaptic component of chemical synapses) was reported to recover following chronic administration of fingolimod (Rossi et al. 2012; Luchtman et al. 2016; Bonfiglio et al. 2017), dimethyl fumarate (Luchtman et al. 2016; Parodi et al. 2015), glatiramer (Gentile et al. 2013) and rituximab (Rossi et al. 2014).

A large part of these studies investigate the effects of the prophylactic administration of the drugs, but some of them also focussed on the therapeutic approach, i.e. the administration of the drug starting from the onset of the first symptoms, a condition well consistent with the timing experienced by MS patients.

In some cases, the possibility was discussed that the drugs could directly affect the release of glutamate, as well as the mechanism of uptake of the endogenous amino acid, by directly modulating neurons and astrocyte functions. Besides the activity at neurons and synaptic connections, however, the beneficial effects of these therapeutics were mainly related to their ability to recover the pathological activation of central glial cells as well as to reduce the infiltration of circulating lymphocytes (both B and T cells) and macrophages in the CNS, claiming for their main immunomodulatory/antiinflammatory activities.

The main targets of fingolimod and derivatives are the sphingosine receptors, that have a wide distribution throughout the CNS, being expressed in neurons, astrocytes, glial cells and oligodendrocytes (Healy and Antel 2016) and that allow to hypothesize either direct and/or indirect activity at neurons/astrocytes to control glutamate excitotoxicity. Chronic prophylactic and therapeutic fingolimod recovers glutamate impairments in selected region of the CNS of EAE mice at different stages of disease (Bonfiglio et al. 2017; Levite 2017; Pittaluga 2017; Rossi et al. 2012). More interestingly, clinical observations unveiled that oral chronic fingolimod restored glutamate-mediated intercortical excitability in patients suffering from the RRMS (Landi et al. 2015).

Differently the efficiency of laquinimod in recovering glutamate excitotoxicity was proposed to rely on a direct control of the uptake efficiency in astrocytes of EAE mice (Gentile et al. 2018). Finally, dimethyl fumarate was found to control glutamate homeostasis in the CNS of EAE mice by causing a change in the molecular and functional phenotype of activated microglia from the classically activated, pro-inflammatory type to the alternatively activated, neuroprotective one. The mechanism of detoxification of astrocytes relied on the activation of the hydroxycarboxylic acid receptor 2 (HCAR2), that, by means of an AMPK-Sirt1 axis, causes deacetylation, and thereby inhibition, of NF-κB-mediated pathways and, consequently, of the secretion of several pro-inflammatory molecules. This neuroprotective effect was exerted on neurons at presynaptic terminals and modulated glutamate release as evidentiated by measuring EPSPs in the cortex (Parodi et al. 2015).

Based on the observation that altered glutamatergic transmission seems to be a hallmark of the onset and progression of the demyelinating disorders, researchers hypothesized the use of glutamate receptors ligands to recover the aberrant glutamatergic transmission. In particular, it was hypothesized the use of NMDA and AMPA antagonists to contain the impact of excitotoxic conditions either on synaptic transmission or on macrophages and lymphocytes recruitment, microglia/astrocytes activation and oligodendrocytes toxicity (Basso et al. 2008; Bolton and Paul 2006; Lim et al. 2017). This approach is of course limited by the lack of safe orally active modulators of the ionotropic glutamate receptors. As far as the NMDA receptors are concerned, the only antagonist available is memantine that is an

uncompetitive NMDA antagonist approved for the therapy of the mild cognitive impairment in Alzheimer's disease and that in recent years has gained interest for the cure of other pathologies (migraine, epilepsy). However, although memantine was reported to provide symptomatic relief to MS patients (Starck et al. 1997), its efficacy was recently revised in the "EMERITE" (NCT01074619) study and the conclusion did not support the use of this drug in MS (Peyro Saint Paul et al. 2016). The EMERITE analysis was dedicated to evaluate the efficacy and safety of the long-term administration of memantine as a symptomatic treatment for cognitive disorders in patients with RR-MS. The results unveiled that memantine administration did not cause significant beneficial effects in the MS patients, but rather that its tolerability was significantly worse than expected. Based on these results, the possibility to approach a direct modulation of NMDA receptors through the administration of receptor antagonists, although attractive, seems unrealistic.

Perampanel is a selective AMPA receptor antagonist that was developed to treat epilepsy. The drug was approved in the USA and Europe to treat localization-related seizures in young and adult patients (Hanada et al. 2011). Studies were dedicated to assess whether perampanel was effective in treating multiple sclerosis, Parkinson's disease, or migraine prophylaxis, but the results showed that the drug was almost ineffective in these pathologies. The development of this drug for the cure of MS was definitively discontinued in 2016.

15.5 Emerging Treatments Related to Glutamate Modulating Drugs for Autoimmune Demyelinating Disease

Although so far there are no mGlu receptors ligands close to approval for entering the clinic (Nicoletti et al. 2011), mGlu receptors (including mGlu2 and 3 receptors) are still considered promising targets for the development of drugs for the treatment of CNS disorders. In particular, data were provided showing that MS patients with cognitive impairment had low hippocampal NAAG levels, suggesting that agonists at mGlu3-preferring receptors might be beneficial in this disease.

Glutamate carboxypeptidase II (GCPII), also known as *N*-acetylated-alpha-linked acidic dipeptidase (NAALADase), is a zinc-dependent peptidase that could represent a target of therapeutic interventions in a variety of neurologic disorders. It is preferentially expressed in astrocytes and Schwann cells (Berger et al. 1995; Sacha et al. 2007). This enzyme cleaves NAAG, inactivating it.

Inhibitors of CGPII are expected to increase the endogenous CNS level of NAAG and therefore to be efficacious in preventing clinical symptoms in MS patients (Rahn et al. 2012).

The first potent and selective GCPII inhibitor, the 2-(phosphonomethyl) pentanedioic acid (2-PMPA), was reported in 1996 (Jackson et al. 1996). 2-PMPA behaves as a competitive inhibitor of GCPII in the picomolar range and it is devoid

of activity at other cellular targets including glutamate transporters and receptors. The administration of 2-PMPA to the EAE mice significantly improved cognition in the animals at the acute stage of disease. To note, the drug was found to be present in the CNS, consistent with a direct central effect. Unfortunately, drugs acting at GCPII for human use with a profile of safety and efficacy are not available (Rahn et al. 2012). We have already discussed the potential use of NMDA antagonists to reduce the synaptic impairments and the excitotoxic events that occur during the development of the demyelinating disorders. We also reported how this approach is limited by the lack of drugs. It is however worth stressing that in recent years another therapeutic approach to modulate the NMDA-mediated signalling is gaining interest. This approach relies on the concept of the "metamodulation" and implies the use of ligands acting at colocalized, functionally coupled receptors, which, either directly or indirectly, control the functions of the receptors they cross-talk with.

In the case of the NMDA receptors, this approach would imply the use of ligands acting at non-glutamatergic receptors that modulate NMDA-mediated responses by controlling the glutamate release in the synaptic cleft or that colocalize and functional cross-talk with the NMDA receptors themselves, controlling their activity. It is the case of the cannabinoid receptors type 1 (CB1) receptors. These receptors exist presynaptically in glutamatergic nerve endings in several CNS regions and their activation negatively controls glutamate exocytosis (Kim and Thayer 2000). Furthermore, data exist showing that CB1 receptors colocalize and functionally couple with NMDA receptors (Neuhofer et al. 2019).

Ligands acting at the CB1 (Manterola et al. 2018; Pryce et al. 2003), as well as the modulators of the enzymatic pathways accounting for the synthesis and/or the metabolism of the endogenous cannabinoids (EC), would be of interest in this approach since they would be expected to tune NMDA-mediated functions.

As far as the MS is concerned, the EC system appears a suitable and challenging target for a therapeutic approach based on a series of evidences:

1. the EC system is deregulated in subjects suffering from demyelinating disorders, as well as in animals suffering from EAE, in line with its role in the onset and progression of the clinical symptoms of the disease (Centonze et al. 2007),
2. despite the above-mentioned deregulations, the EC system can function as a tuning system that could mediate the restoration of neuronal and astrocyte impairment (Pryce et al. 2003, 2015),
3. beside the control of central transmission, the EC system also modulates inflammatory processes involved in the pathological course of the demyelinating disorders (Rossi et al. 2011, 2015).

The evidence supports the interest around the discovery of drugs able to modulate the EC system for the cure of MS. The data so far available from clinical studies, however, indicate that cannabis-based medicines have a narrow therapeutic window, in particular because of the CB1 receptor-mediated psychoactive components of the drug(s) used in therapy (Parmar et al. 2016). Unfortunately, the possibility to avoid this CB1-mediated effect favouring the other CB1/cannabinoid receptors type 1 (CB2)-mediated activities by using broad spectrum exogenous receptor agonists

seems unlike and the probability that these unwanted events occur during treatment with cannabis derivatives or cannabinoid-like molecules is very high.

The advance in elucidating the enzymatic pathway accounting for the synthesis and the metabolism of ECs, however, has recently determined an important progress in cannabis-mediated medicine. In particular, the possibility to increase the levels of the ECs by the pharmacological inhibition of the enzymes involved in their degradation has emerged as a valuable approach that could represent a safe alternative way to strengthen the cannabinoid-mediated control of both central neurotransmission and inflammatory pathways (Lourbopoulos et al. (2011) and reference therein). Accordingly, the modulation of these enzymatic pathways may allow a fine-tuning of receptor-mediated functions, thus reducing the possibility of concomitant effects due to CB over-activation.

Two are the main ECs in the CNS, i.e. anandamide and 2-arachidonoylglycerol (2-AG). Anandamide is mainly metabolized by the well-characterized fatty acid amide hydrolase (FAAH). FAAH selective inhibitors enhance anandamide levels and induce analgesia and anxiolytic effects. However, the analgesic effects due to the irreversible inhibition of FAAH have not been replicated in phase II clinical studies and, more importantly, a phase I study on a FAAH inhibitor recently failed because of serious lethal side effects (Bonifácio et al. 2020).

Alternative to the blockade of FAAH is the inhibition of the 2-AG catabolic enzymatic pathway, the monoacylglycerol lipase (MGL). This enzyme is responsible for about 85% metabolism of 2-AG, the major EC in the CNS, which acts as a full agonist at both CB1 and CB2, with lower potency than anandamide towards the CB1/CB2 receptors. It has been suggested that MGL inhibitors could be useful for the treatment of several diseases, including pain, neuropsychiatric disorders, cancer and neurodegenerative disorders like MS. Evidence showing that the blockade of this enzyme can elicit any adverse effect is so far lacking. Moreover, some recent findings suggest that MGL inhibition can be attained without the onset of concomitant undesirable side effects mediated by central CB1 (Anderson et al. 2014). As the demyelinating diseases are concerned, data in literature demonstrated that enzyme inhibitors acting at both FAAH and MGL are active in in vivo studies in the EAE mouse model of MS, in which they clearly ameliorated the course of disease without inducing unwanted effects linked to the CB1 overstimulation (Bernal-Chico et al. 2015; Brindisi et al. 2016; Hernández-Torres et al. 2014).

It is predicted that the administration of the MGL inhibitors would be expected to increase the tuning of the endogenous cannabinoid at CB1 and CB2 receptors, including those located presynaptically that control glutamate release efficiency (Musella et al. 2014; Sánchez-Zavaleta et al. 2018), as well as those colocalized with the NMDA receptors, whose activation "metamodulates" the colocalized glutamatergic receptors (Neuhofer et al. 2019).

Finally, a new approach has been proposed to control the hyperglutamatergicity that typifies several central neuropathologies, including MS, the so-called blood glutamate scavenging approach. When glutamate concentrations are pathologically elevated in the brain, several inherent mechanisms can participate to reduce its level. One such mechanism utilizes sodium-dependent transporters located on brain

capillaries that provide an important way by which pathologically glutamate is reduced in the brain and diffuses in the blood through mechanisms of facilitated diffusion (Zhumadilov et al. 2015). The rate of brain-to-blood glutamate efflux can be increased (Gottlieb et al. 2003) by using the blood enzymes glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT), which, in the presence of their cosubstrates pyruvate and oxaloacetate, convert glutamate to 2-ketoglutarate. By injecting pyruvate and oxaloacetate in the peripheral blood, Gottlieb and colleagues successfully increased the rate of elimination of glutamate from the brain ECF, providing the first demonstration that the manipulation of blood glutamate dramatically reduces brain glutamate concentrations.

The clinical relevance of the preclinical results concerning this approach has been revised by Castillo et al. (2016). The results from clinical studies are in progress and, if positive, would allow the use in therapy of this approach to contain hyperglutamatergicity in patients.

15.6 Conclusion and Future Perspectives

Based on the findings revised in the chapter, it seems conceivable to confirm the main role of the glutamatergic system in the aetiopathogenesis of MS. The results concerning the role of glutamate derangement, either in the dysregulation of the immune system or in determining the impaired chemical transmission at central synapses, that were once obtained in MS animals models were largely confirmed in MS patients. These observations proved the main role of glutamate in controlling the immunocompetent responses, but also highlight its impact on the development of the synaptic derangements that typify the demyelinating disease.

Very interestingly, several therapeutics known to modulate the immunocompetent responses were recently reported also to significantly recover the glutamatergic central derangements both in EAE mice and in MS patients. These observations by one side further support the strict correlation and functional interaction linking the IS and the CNS, but also suggest new approaches to counteract central excitotoxicity. The efficacy of certain drugs (fingolimod, laquinimod) to recover glutamate transmission might open the road to the use of these therapeutics also for the cure of other central pathologies that are typified by overt altered glutamate homeostasis. These observations are particularly intriguing, if one considers that almost all the glutamate receptor ligands (with few exceptions, see for instance memantine) that were proposed as promising drugs for the cure of central disorders were discontinued because of the onset of unwanted side effects.

Alternative to the use of glutamate receptor ligands, some preclinical results in EAE mice also unveiled the efficacy of alternative approaches that rely on the use of indirect modulators of the glutamatergic system. It is the case the GCPII inhibitors, which would modulate the bioavailability of endogenous glutamate ligands at selective receptor subtypes. In this context, a particular attention must be paid to the repositioning of therapeutics that are currently in use for certain pathologies but

that can "metamodulate" the functions and the expression of glutamate receptors and might assure an indirect control and tuning of glutamate transmission in the CNS. We are referring to the MGL inhibitors that would "metamodulate" glutamate receptors colocalized with cannabinoid receptors, restoring their physiological role in CNS. All these approaches however deserve further investigations to translate them to clinical studies and to ascertain their safety, tolerability and efficacy.

Finally, the new approach of blood glutamate scavenging represents a revolutionary therapeutic strategy to be evaluated for its efficacy in containing hyperglutamatergicity in MS patients.

We firmly believe that in the next future the study of the impact of the available therapeutics on central glutamatergic system would improve our knowledge of the mechanisms underlying the onset and the progression of MS, also unveiling new cellular/molecular targets of new therapeutics.

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Suggested Reading

Bolton C, Paul C (2006) Glutamate receptors in neuroinflammatory demyelinating disease. Mediat Inflamm 2006:93684. The review considers the relevance of the glutamate receptors in EAE and MS pathogenesis, focussing particularly on the ionotropic receptors. The use of receptor antagonists/ receptor modulators to control EAE is also discussed together with the possibility of their therapeutic application in demyelinating disease
Klaver R, De Vries HE, Schenk GJ et al (2013) Grey matter damage in multiple sclerosis: a pathology perspective. Prion 7:66–75. Starting from evidence obtained in the last decade from immunohistochemical studies showing that grey matter (GM) pathology in multiple sclerosis is extensive, the authors focussed on the results obtained in the last decade with magnetic

resonance imaging technique which unveiled the GM damage is present from the earliest stages of the disease and accrues with disease progression. These observations support the conclusion that GM pathology is clinically relevant as it correlates with multiple sclerosis associated motor deficits and cognitive impairment

Mandolesi G, Gentile A, Musella A et al (2015) Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis. Nat Rev Neurol 11:711–724. The review resumes the work of the group headed by Diego Centonze concerning the role of synaptic alterations in the onset and the development of multiple sclerosis. Based on the observations supporting the pathological impact of proinflammatory cytokine on synaptic efficiency, including the glutamate-GABA cross-talk, the authors propose the term synaptopathy to describe the complex interaction linking the immune system and the CNS in the course of the disease

Di Filippo M, de Iure A, Durante V et al (2015) Synaptic plasticity and experimental autoimmune encephalomyelitis: implications for multiple sclerosis. Brain Res 1621:205–213. Starting from the concept that synaptic plasticity assures the ability of the CNS to cope with injuries in an adaptive or maladaptive manner, the authors discuss the impact of peripheral and central inflammation on neuroplasticity in the course of multiple sclerosis from this point of view. By reviewing the available literature concerning the synaptic derangements in the course of the experimental autoimmune encephalomyelitis in mice, the review aims at deciphering the complex pathways involved in the progression of the disease, discussing their relevance to clinical outcomes

Danbolt NC, Furness DN, Zhou Y (2016) Neuronal vs glial glutamate uptake: resolving the conundrum. Neurochem Int 98:29–45. The review deals with excitatory amino acid transporters (EAATs) in the central nervous system, particularly focussing on the transporters that are expressed in neurons and in astrocytes, on their role in controlling glutamate bioavailability and their main regional and cellular distribution. The manuscript tackles with emphasis the role of EAAT on the glutamate-glutamine cycle as well as on the mechanism of heteroexchange compared to net uptake. Finally, the review also discusses the role of EAAT in controlling glutamate releasing probability

Levite M (2017) Glutamate, T cells and multiple sclerosis. J Neural Transm 124:775–798. The review offers a wide overview of the involvement of the glutamatergic system in the onset and the development of demyelinating disorders, particularly focusing on the pathological cross-talk linking glutamate and immune system. This is an interesting review that describes item by item the literature available on the events accounting for glutamatergic derangements in MS

Stampanoni Bassi M, Mori F, Buttari F et al (2017) Neurophysiology of synaptic functioning in multiple sclerosis. Clin Neurophysiol 128:1148–1157. The review focusses on the pathological relevance of the cross-talk linking the central nervous system and the immune system in the development and the progression of multiple sclerosis starting from the results from animal models to the clinical studies supporting the functional link between alterations of central transmission in MS patients in relation to different phenotypes and disease phases. The review also deals with explorative studies on neuronal plasticity in MS patients using the transcranial magnetic stimulation. Emphasis is dedicated to the pathological overproduction of proinflammatory cytokines and chemokines and neuronal survival and neurological manifestations in MS patients, owing to support the main involvement of inflammatory-driven, synaptic dysfunctions in the development of MS

Fazio F, Ulivieri M, Volpi C et al (2018) Targeting metabotropic glutamate receptors for the treatment of neuroinflammation. Curr Opin Pharmacol 38:16–23. The review provides a synthetic and current overview of the role and the involvement of metabotropic glutamate receptors in the onset and development of demyelinating disorders in animals as well as in MS patients

University of California, San Francisco MS-EPIC Team, Cree BAC et al (2019) Silent progression in disease activity-free relapsing multiple sclerosis. Ann Neurol 85:653–666. Based on clinical data the authors introduce the term “silent progression” to describe the insidious disability that accrues in many patients who satisfy traditional criteria for relapsing–remitting MS. In particular

the authors provide evidence that the silent progression during the RRMS phase is associated with brain atrophy suggesting that the same process that underlies SPMS likely begins far earlier than is generally recognized. This conclusion supports a unitary view of MS biology, with both focal and diffuse tissue destructive components, and with inflammation and neurodegeneration occurring throughout the disease spectrum playing a role in the disease development

Chapter 16
The Role of Glutamate Dysregulation in the Etiology of ADHD

P. E. A. Glaser, S. R. Batten, and G. A. Gerhardt

Abstract In this chapter we review current data assessing the role of glutamate in the etiology of ADHD. A general introduction of ADHD and common comorbidities are briefly discussed. The glutamate system in general and its potential role in ADHD are thoroughly reviewed evaluating both preclinical and clinical data. The current ADHD treatments that act on the glutamate system, memantine and atomoxetine, are discussed. The chapter concludes with a discussion on the future of glutamatergic drugs in the treatment of ADHD.

Keywords ADHD · Brain energetics · Glutamate · Glutamatergic pharmacotherapies

16.1 Introduction

Attention-deficit hyperactivity disorder (ADHD) is a disorder commonly diagnosed in children and adolescents that begins in childhood and may often continue into adulthood (Martins et al. 2014; Granet et al. 2005). ADHD is considered a neurodevelopmental disorder that is characterized by deficits in attention and impulse control, often accompanied by hyperactivity (Martins et al. 2014; Morgan et al. 1996). Currently, the etiological processes of ADHD are not well understood despite the fact that it affects 5% of children and 2.5% of adults worldwide (American Psychiatric Association 2013).

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_16

Historically speaking, the earliest reference of ADHD or a "disease of attention" is rumored to be by Shakespeare in his play *King Henry VII* (Martins et al. 2014). However, a better-documented report was in 1902 by the English physician, George Still, who focused on clinical aspects of those with ADHD-like symptoms and argued that there were certain traits similar in all those who seemed to have this "disorder" (Mash and Barkley 2003). Through the years ADHD has had several different nomenclatures and diagnostic criteria (Martins et al. 2014). Currently, the DSM-5 denotes three presentations of ADHD including inattentive (ADHD-PI), hyper-active/impulsive (ADHD-HI), and combined (ADHD-C) (American Psychiatric Association 2013). The DSM-5 also established more "adult relevant" symptoms as well as the classification system for symptoms as mild, moderate, and severe (American Psychiatric Association 2013).

Regardless of the nomenclature used throughout the years, it has been hypothesized for decades that ADHD is a heterogeneous disorder involving interactions between genetics and the environment (Genro et al. 2012; Biederman 2005). Environmentally speaking, positive correlations are observed between ADHD and lower socioeconomic status, parent criminality, parental mental health, fetal exposure to alcohol, and maternal smoking as well as many other factors (Martins et al. 2014; Shimizu and Miranda 2012; Zhang et al. 2012). From a genetic standpoint most genes associated with ADHD seem to be related to malfunctions in several neurotransmitter systems with the most studied being those associated with the dopaminergic, noradrenergic, and serotonergic systems (Zhang et al. 2012; Roman et al. 2002; Kirley et al. 2002; Faraone et al. 2001).

More recently, however, evidence suggests that genetic and neurobiological issues related to the glutamate system may be a contributing factor in the etiology of ADHD (Miller et al. 2014; Miller et al. 2013; Reif et al. 2009; Hoogman et al. 2011). Here we will outline comorbidities associated with ADHD as well as discuss the glutamate system in general, review data supporting the role of glutamate in ADHD, discuss current glutamatergic pharmacotherapies, and discuss future perspectives on glutamatergic drugs in meliorating the symptoms of ADHD.

16.2 ADHD Comorbidities

In a study of children and adolescents referred for ADHD, comorbidity ranged from ten to fifty percent for other common psychiatric disorders of youth (Rohde et al. 2004). Another study found roughly 62% of ADHD patients are likely to have one or more comorbidities and 35% of patients are likely to have two or more psychiatric comorbidities (Yoshimasu et al. 2012). However, no differences in comorbidities seem to exist between males and females diagnosed with ADHD (Yoshimasu et al. 2012).

The main psychiatric conditions that accompany ADHD include disruptive behavior disorders (30–50%), depression (15–20%), anxiety disorders (25%), learning disabilities (10–15%), and substance abuse disorders (9–40%) (Rafolovich

2001). Of the disruptive disorders, a strong association is seen with ODD/CD (Yoshimasu et al. 2012). Also, those with comorbid ADHD and major depression are more likely to develop bipolar disorder later in life (Chen et al. 2015).

There is no difference in comorbidity rate between males and females with ADHD; however, there are differences in the type of diseases comorbid with ADHD between the sexes (Yoshimasu et al. 2012). Specifically, males are more likely to have externalizing-only disorders (i.e., ODD/CD) and females internalizing-only disorders (i.e., anxiety) (Yoshimasu et al. 2012). Thus, ADHD is a disorder that is likely to be accompanied by a myriad of other disorders. These comorbid data should be considered when thinking of the glutamatergic system's role in ADHD and how the glutamate system may promote other psychiatric disorders.

16.3 ADHD and the Glutamate System

Several studies have shown issues with the prefrontal cortex (PFC) as well as other "higher level" circuits in the brain in those with ADHD (Carrey et al. 2002; Moore et al. 2006; Moore et al. 2007). Considering the many glutamatergic projections to the PFC as well as other cortical regions it is no surprise that researchers have begun exploring the glutamate system in those with ADHD (Moore et al. 2007). Glutamatergic changes in human ADHD have also stimulated preclinical work on glutamate and ADHD (Miller et al. 2013; Miller et al. 2019). Thus, it is becoming apparent that dysregulations in the glutamate system may be one key etiologic factor in ADHD. In this section we will examine general aspects of the glutamate system as well as potential glutamatergic dysfunctions seen in ADHD.

16.3.1 Glutamate System Basics

Glutamate is the major excitatory neurotransmitter in the CNS (Danbolt 2001). Glutamate is synthesized in the nerve terminals of glutamatergic neurons from two major sources: α-ketoglutarate produced from the TCA cycle or from glutamine shuttled to neurons from glial cells (Tapiero et al. 2002; Anderson and Swanson 2000; Daikhin and Yudkoff 2000). In neuron terminals α-ketoglutarate and glutamine are enzymatically synthesized into glutamate and packaged into synaptic vesicles in an energy dependent fashion via vesicular glutamate transporters (VGLUT) (Fonnum et al. 1998).

Once packaged, glutamate can then be released into the synaptic cleft, in a Ca^{2+} dependent manner, upon the firing of an action potential (Meldrum 2000; Turner 1998). Once glutamate is released it is free to: (1) bind to pre- and post-synaptic receptors, (2) be taken up by glial cells in a Na^{+} dependent fashion, (3) be actively transported into presynaptic neurons and repackaged, and finally, (4) diffuse away

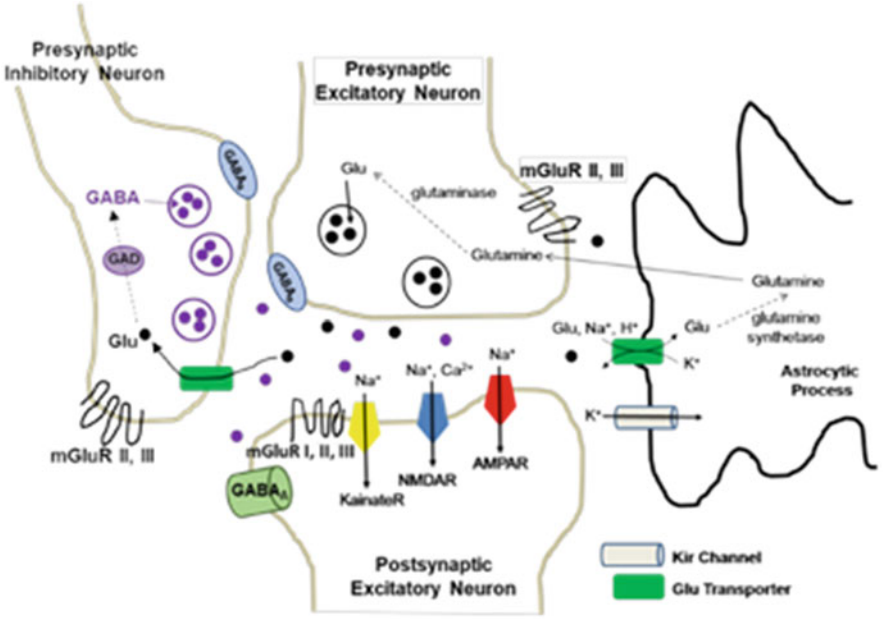

Fig. 16.1 A simplified glutamatergic synapse showing interactions from glial cells and GABA interneurons. Once glutamate is released from the presynaptic neuron it is free to bind pre- and post-synaptic ionotropic and metabotropic receptors or be taken up by high affinity EAATs on glia cells in a Na^+ dependent fashion. Binding to AMPA, NMDA, Kainate, or mGluR1 receptors will cause excitation. Binding to mGluR2,3 receptors causes cellular inhibition. GABA downregulates the Glutamate synapse at GABA-A and GABA-B receptors

from the synapse (Anderson and Swanson 2000; Daikhin and Yudkoff 2000; Attwell 2000).

In the synaptic cleft glutamate may bind to ionotropic receptors (NMDA, AMPA, Kainate), presynaptic inhibitory metabotropic receptors (mGluR2, 3, 4, 7, 8), and/or post-synaptic excitatory metabotropic receptors (mGluR1, 5) (Meldrum 2000; Iversen et al. 2009; Schoepp et al. 1998). Approximately 90% of glutamate is taken up by astrocytes either by excitatory amino acid transporter 1 or 2 (EAAT 1 and EAAT2, respectively) (Iversen et al. 2009; Schoepp et al. 1998; Danbolt et al. 1998). Glutamate can also be taken up by neurons via EAAT3–5 although this seems to be a secondary process for this neurotransmitter system (Iversen et al. 2009; Schoepp et al. 1998; Danbolt et al. 1998). See Fig. 16.1 for a simplified diagram of the glutamate synapse, astrocyte uptake, and the regulation of glutamate signaling by GABA interneurons.

16.3.2 Bridging Old and New Thoughts About Neural Signaling in ADHD

Researchers became further interested in glutamate dynamics in the PFC in those with ADHD after realizing the reciprocal modulatory nature between these glutamatergic circuits and the dopamine system (the system historically most widely studied in ADHD) (Miller et al. 2013). Specifically, glutamate projections from the PFC project to dopamine rich areas such as the striatum, nucleus accumbens, ventral tegmental area (VTA), and substantia nigra (Iversen et al. 2009; Schultz 2001). Dopamine projections from the VTA and nucleus accumbens also project to the PFC (Iversen et al. 2009; Schultz 2001). Thus, malfunctions in either of these systems could cause dysregulation(s) in the others (Miller et al. 2013).

Studies have shown that the NMDA receptor is crucial in stimulating dopamine neurons in the VTA and substantia nigra (Martinez-Fong et al. 1992; Warton et al. 2009). Also, there is evidence that D2 receptor activation may inhibit the excitatory effects of NMDA receptors (Kotecha et al. 2002). Similarly, D4 receptor activation may decrease the excitatory response of AMPA receptors in PFC pyramidal neurons (Yuen et al. 2010). Thus, glutamatergic dysregulation in ADHD may be as detrimental as those seen in the dopamine system and therefore normalization of this system may help alleviate symptoms seen in ADHD.

16.3.3 ADHD and Glutamate: Evidence from Human Studies

Decreased functioning of the PFC and subsequent deficits in cognitive functioning including working memory have been observed in those with ADHD (Castellanos and Tannock 2002; Tannock et al. 1995). An MRI study has shown that there is an increase in a marker for glutamate in the anterior cingulate cortex of those with ADHD compared to controls (Moore et al. 2007). There is also evidence that treating children with medications known to meliorate the symptoms of ADHD decreases glutamate levels in the striatum and PFC (Hammerness et al. 2012). A spectroscopic study of children with ADHD showed decreased concentration of glutamate ($p = 0.009$), N-acetyl aspartate (NAA) ($p = 0.029$) and choline ($p = 0.016$) in ADHD participants compared to controls specifically in the right striatum and no significant changes in the left striatum (Hai et al. 2020). Genetic associations have been found supporting the involvement of GABAergic and Glutamatergic systems in ADHD when there is overlap of symptoms with ASD (Autism Spectrum Disorder) (Naaijen et al. 2017). While this research is promising, there is no robust clinical data on the role of glutamate in ADHD. However, more data from preclinical models are suggestive of glutamate dysfunction in this disorder.

16.3.4 ADHD and Glutamate: Evidence from Animal Research

The spontaneously hypertensive rat (SHR) is the most widely used model of ADHD specifically for studying the ADHD combined type (Sagvolden and Johansen 2011; Russell 2001a). The SHR shows attention deficits, hyperactivity, and impulsivity in motor movements (Sagvolden 2000; Sagvolden et al. 1992; Knardahl and Sagvolden 1979). While this model has been widely used in studying ADHD pathology, there are recent arguments about how well this animal model translates to the human disease, what animal models may represent other subtypes, and what the appropriate control should be in the experiments (Alsop 2007). That being said, most agree that the SHR from Charles River (SHR/NCrl) is the most appropriate animal model for ADHD-C, the Wistar-Kyoto from Charles River (WKY/NCrl) is best suited as a model for ADHD-PI (predominately inattentive type), and that the WKY from Harlan (WKY/NHsd) is the most appropriate control with the outbred Sprague-Dawley (SD) being another potential control (Russell 2001a; Sagvolden et al. 2005, 2008, 2009). Several different studies have shown glutamate dysregulation in these animals.

Studying glutamate dynamics, specifically in the rat equivalent of the human PFC, seems to be particularly important in understanding the etiology of ADHD (Carrey et al. 2002; Moore et al. 2006, 2007). AMPAR-mediated synaptic transmission in pyramidal neurons of PFC was diminished in SHR, which was correlated with the decreased surface expression of AMPAR subunits (Cheng et al. 2017). Evidence from our lab has shown a significant increase in KCl-evoked glutamate release in regions of the PFC, the cingulate and infralimbic cortices, in the SHR compared to the WKY (Miller et al. 2019). It is worth noting that evidence suggests that the cingulate cortex may regulate emotions in humans as well as be a primary center for motivation (Granziera et al. 2011; Adey and Meyer 1952). On the other hand, the infralimbic region seems to be related to attentional focus as well as attentional set-shifting (Dalley et al. 2004). Note that these aforementioned issues are all seen in ADHD (Krusch et al. 1996; Mehta et al. 2004; Klimkeit et al. 2005).

Data collected also suggest that glutamate release may be increased in the striatum of the SHR compared to the WKY (Miller et al. 2019). This finding may relate to ADHD in that the striatum is related to movement and reward circuitry both of which seem to be disrupted in ADHD (Dunnett and Lelos 2010; Salamone and Correa 2012). It also takes a significantly higher volume of ejected glutamate to achieve similar peak amplitudes in SHR rats compared to WKY control suggesting uptake may be faster in the PFC of SHRs; this may be a potential compensatory response to increased vesicular glutamate release in this area (Miller et al. 2019). Considering the differences seen above in anesthetized rats our lab has also attempted to assess glutamate dynamics in freely-moving animals.

Evidence suggests that tonic glutamate concentrations are higher in SHR animals compared to WKY rats regardless of treatment with methylphenidate (Miller et al. 2019). Specifically, higher tonic levels were seen in the cingulate, prelimbic, and

infralimbic cortices compared to WKY (Miller et al. 2019). Similarly, tonic glutamate levels are increased in the PFC of both intermediate and chronically treated methylphenidate animals compared to SHR saline controls (Miller et al. 2019). Further, phasic glutamate was decreased in SHR rats treated chronically with methylphenidate compared to SHR rats treated with saline (Miller et al. 2019).

It is worth noting that the type of phasic signal seen in the animals in this experiment differed between strain and treatment (Miller et al. 2019). Specifically, SHR's treated intermediately with methylphenidate had more rapid, multi-peaks compared with WKY's treated with methylphenidate (Miller et al. 2019). However, when treated chronically with methylphenidate, the SHR rats had more slow phasic events compared to SHR saline controls (Miller et al. 2019). Overall, these freely-moving data suggest that there are dynamic changes in both tonic and phasic glutamate levels in the PFC of SHR treated with a well-known dopamine acting drug often prescribed to those with ADHD.

More "indirect measures" have also shown differences in glutamate signaling in the SHR. For example, there is evidence that NMDA receptor activation resulted in less calcium influx in SHR PFC slices compared to those obtained from WKY (Lehohla et al. 2004). Glutamate applied to SHR PFC slices also showed an increase in norepinephrine release by activation of NMDA receptors compared to WKY controls (Russell 2001b).

Increased glutamate simulated release of dopamine in the substantia nigra of SHR rats compared to WKY controls has also been found suggesting that there may be altered regulation of dopamine by glutamate in the SHR (Warton et al. 2009). Mice with inactivation of the dopamine transporter showed increased hyperactivity when administered NMDA antagonists and the hyperactivity decreased when given drugs that increased glutamate signaling (Gainetdinov 1999; Gainetdinov 2000). Neonatal 6-hydroxydopamine lesions in rats caused dose dependent decreases in D4 receptors and increases in glutamate transporters in the striatum (Masuo et al. 2002). All of this data further suggests tightly coupled interactions between the glutamate and dopamine systems in ADHD (Masuo et al. 2002).

Inactivation of mGluR5 increases hyperactivity in mice (Halberstadt et al. 2011). Further, impairment of presynaptic mGLuR7 receptors using MMPIP in the prelimbic cortex decreased visuospatial attention (Benn and Robinson 2014). However, in this same study no other NMDA antagonists used or mGluR2/3 inhibitors caused any changes in impulse control when infused into the prelimbic or infralimbic areas (Benn and Robinson 2014). Nevertheless, considering that mGluR drugs may work to normalize glutamatergic signaling, agonists of these receptors may normalize the glutamatergic system and behavior in these animals (Li et al. 2014). Along with this, downstream effectors of mGluRs may be dysregulated in ADHD as well.

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) signaling in the PFC in glutamatergic neurons may also contribute to ADHD symptomology. CaMKII and PKC are essential for long-term potentiation (LTP); a process highly associated with glutamatergic signaling. In fact, it was found that an increase in CaMKII autophosphorylation and GluR1 phosphorylation was

found in the mPFC of stroke prone spontaneous hypertensive rats (SHRSP) compared to WKY controls suggesting mPFC pathology; this was related to impaired performance on both the Y maze task and a novel discrimination task (Yabuki et al. 2014). Furthermore, methylphenidate treatment did not only increase SHRSP performance on behavioral tasks but also decreased CaMKII autophosphorylation and GluR1 phosphorylation in the mPFC compared to WKY controls (Yabuki et al. 2014).

Using the 5-choice serial reaction time task (5CSRTT) it has been reported that NMDA antagonism by MK-801 in the infralimbic cortex increased impulsive responding (Benn and Robinson 2014). In another study assessing the role of the glutamate system in impulsive choice (delay and probability discounting) it was found that systemic injections of MK-801 decreased discounting rate for the larger reinforcer (less impulsive) (Yates et al. 2015). These discrepancies may be due to the different underlying processes thought to contribute to impulsive action compared to impulsive choice (Perry and Carroll 2008). Regardless, these data do suggest that some kind of interaction is occurring with the glutamate system in models of impulsivity although from these studies the directionality is not clear.

16.3.5 Glutamate, ADHD, and Genetic Correlates

Latrophilin-3 (LRHN3), an adhesion G-protein coupled-receptor indicated in synaptogenesis and synaptic plasticity, has been shown to promote ADHD-like behaviors in experimental models with loss-of-function mutation in the *LRHN3* gene (Silva et al. 2011). Specifically, LRHN3 knockdown causes increased locomotor activity and dopamine signaling and can be rescued by drugs used to treat ADHD (Lange et al. 2012).

Fibronectin (FLRT3) is a transmembrane protein and a natural ligand of LRHN3; this binding is essential to glutamatergic signaling (O'Sullivan et al. 2012). When FLRT3 and LRHN3 bind this action seems to regulate excitatory synapses and plasticity both pre- and post-synaptically (Yamagishi et al. 2011). Some with ADHD have been shown to contain mutations in FLRT3 further suggesting links between glutamate signaling and ADHD (Lionel et al. 2011).

Mutations resulting in malfunctioned SHANK proteins may also cause ADHD symptoms (Durand et al. 2007). SHANK proteins are synaptic multi-domain scaffold proteins of the post-synaptic density that connect receptors, ion channels, and other types of membrane proteins to actin cytoskeleton G protein-coupled pathways involved in dendrite maturation and synapse formation (Lesch et al. 2008). Gene mutations in nitric oxide synthase-1 (NOS-1), a protein closely associated with the NMDA receptor and responsible for nitric oxide generation, have been linked to ADHD behaviors and impulsivity as well (Hoogman et al. 2011; Reif et al. 2006).

Mutations in several other genes including those that code for NMDA receptor subunit-2A and 2B as well as genes encoding for glial glutamate transporter EAAT1

and mGluRs have all been shown to be associated with ADHD (Lesch et al. 2008; Dorval et al. 2007; Turic et al. 2005; Elia et al. 2012).

16.3.6 Brain Energetics and Glutamate: A New Way to View ADHD?

The brain is an interesting organ in the fact that it is about 2% of a human's mass but utilizes approximately 20% of its oxygen and glucose (Magistretti et al. 1999). Further, unlike other organs in the body the brain can only use sugars for energy due to the blood-brain barrier (Fonseca-Azevedo and Herculano-Houzel 2012). Neurons are also interesting in the fact that they are mercurial in nature; they can go from quiescence to rapid firing in seconds (Attwell and Laughlin 2001). Even more interesting is that neurons cannot store their own energy; this is a task left to glia that store energy in the form of glycogen (Almeida et al. 2001). While these aforementioned facts are generally accepted the molecule used as the primary energy source in the brain is still debated (Barros 2013).

It is a well-accepted scientific fact that most of the body uses glucose as its primary energy source. While the brain does seem to use glucose to some degree evidence suggests that lactate may be used more frequently as an energy source by the brain (Barros 2013). This balance and shuttling of glucose and lactate in the brain is tightly performed and regulated by neuron-glia coupling (Magistretti 2006).

Glucose is transported into the brain through blood vessels and into astrocytes via glucose transporters (GLUT1) (Magistretti 2006). Glucose is then transformed into glucose-6-phosphate then either enzymatically transformed into glycogen via glycogen synthase or turned into pyruvate then lactate via pyruvate kinase and lactate dehydrogenase, respectively (Barros 2013; Magistretti 2006). Lactate can then be shuttled out of astrocytes via monocarboxylate transporter 4 (MCT4) and shuttled into neurons via MCT2 (Barros 2013; Magistretti 2006). Once lactate is in neurons it is then transformed back into pyruvate and taken up by mitochondria for use in the TCA cycle (Fig. 16.2) (Barros 2013; Magistretti 2006).

It has been shown that there is tight coupling between glutamate signaling and lactate shuttling (Magistretti 2006). Evidence suggests that when glutamate is released from neurons and taken up by glia via EAATs that this Na^+ dependent process causes lactate to be released to neurons to further support their firing (Magistretti 2006). Also, memory formation, a process highly associated with glutamate, also seems to be associated with lactate (Newman et al. 2011; Suzuki et al. 2011). Research shows that blocking lactate transport in the brain may inhibit memory formation (Newman et al. 2011; Suzuki et al. 2011). Also, lactate but not glucose could "rescue" this loss of memory (Suzuki et al. 2011). Further, evidence suggests that beyond lactate's inherent energy properties it may also serve as a second messenger for potentiating LTP likely by regulating the redox state of neurons (Yang et al. 2014). Considering this research, there is ample evidence to

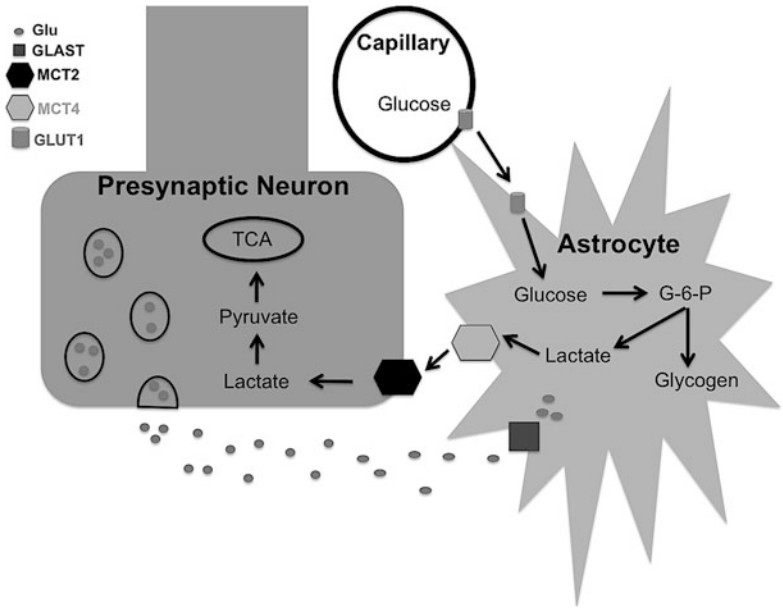

Fig. 16.2 The Astrocyte Neuron Lactate Shuttle. Glucose is transported into the brain through blood vessels and into astrocytes via glucose transporters (GLUT1). Glucose is then transformed into glucose-6-phosphate (G-6-P) then either transformed into glycogen or into lactate. Lactate can then be shuttled out of astrocytes via monocarboxylate transporter 4 (MCT4) and shuttled into neurons via monocarboxylate transporter 2 (MCT2). Once lactate is in neurons it is then transformed into pyruvate and taken up by mitochondria for use in the TCA cycle

suggest that the dysregulation in brain energy can directly affect glutamate signaling. Thus, insufficiencies in how the brain acquires energy could directly affect neurotransmitter systems and promote disease.

Energetics and astrocytic control of glucose was proposed as a possible etiology for ADHD by a review paper in 2001 (Todd and Botteron 2001). More recently some research has shown that lactate hypo-function in the brain can cause aberrant neuronal firing promoting the symptoms seen in ADHD (Killeen et al. 2013). In their elegant paper Killeen et al. (2013) proposed the behavior neuroenergetics theory of ADHD. The neuroenergetics theory assumes brain lactate hypo-function in those with ADHD and uses the lactate-neuron shuttle as a "bottle neck" in neuronal energy acquisition. Using the neuroenergetics theory, with the help of the neuroenergetics mass-action model, these authors derived equations tying neuron physiology to behavior (Killeen et al. 2013).

This model inferred that those with ADHD could only bring approximately 75–80% of their neurocognitive energy to exhibit on tasks (Killeen et al. 2013). Further, ADHD individuals could only allocate approximately 85% of their cognitive resources to behavioral tasks compared to controls (Killeen et al. 2013). Even more impressive than the aforementioned was that parameters derived from the model predicted performance on behavioral tasks suggesting that brain lactate levels

are predictive of behavioral outcomes (Killeen et al. 2013). This fact alone suggests hypo-energetics and changes in glutamate signaling may be very important in the etiology of ADHD.

Other researchers have also found a link between brain energetics, neurotransmitter dysregulations, and ADHD. For example, MRI studies suggest that the ability of those with ADHD to "summon" glucose for oxidative metabolism is impaired in the fronto-parietal system compared to typically developing controls (Cortese et al. 2012). Further, some data support that those with ADHD may have enzyme deficiencies related to energy supply and that these deficiencies may cause developmental delays in the fronto-striatal circuitry (Fair et al. 2010). Evidence also suggests that those with ADHD may require more brain energy than controls to function due to inefficient neural networks lacking full myelination (Nagel et al. 2011).

Dysfunctional GABAergic fibers are also implicated in promoting impulsivity in those with ADHD due to lack of these interneurons to inhibit upper motor neurons (Koos and Tepper 1999). This GABAergic dysfunction may be caused by lack of energy supply; this is thought to be especially relevant when considering that fast-spiking GABAergic neurons may fail to completely inactivate their sodium channels upon action potential firing thus allowing two times the amount of sodium to enter the cell (Carter and Bean 2009). This larger intake of sodium then increases the ATP needed for GABA cells to adequately restore membrane potential, thus this may make these cells especially vulnerable to energy insufficiencies and thus be a potential link in ADHD etiology (Killeen et al. 2013).

NAA levels, a neuro-specific energy storage molecule important for energy metabolism and myelin sheath formation, is altered in ADHD (Ariyannur et al. 2010; Yang et al. 2010; Perlov et al. 2009). Evidence suggests that treatment with methylphenidate increased NAA levels in the anterior cingulate cortex in those with ADHD (Kronenberg et al. 2008). Considering that the anterior cingulate cortex is essential in error processing and learning from mistakes (a deficit seen in those with ADHD) this increase in the energy metabolite NAA may be crucial to ADHD symptoms (Van Meel et al. 2007). The implication of NAA levels in ADHD etiology is even more pronounced when we again consider that neurons in the ADHD brain may be slower processing, more energy insufficient, and variable due to lack of myelination (Nagel et al. 2011; Harris and Attwell 2012; Zhu et al. 2012).

It is worth noting that there is evidence against lactate as an energy source for neurons, glutamate-lactate coupling, and energy deficits in those with ADHD (Jolivet et al. 2010; Pancani et al. 2011). However, considering the overwhelming evidence for a relationship between energy dynamics, glutamate, and ADHD it is likely that issues with brain energy may be a primary cause of the disease and perhaps even a potential target of future medications.

16.4 Targeting the Glutamate System to Treat ADHD

Recently studies have suggested that not only may the glutamate system promote ADHD pathology but also that targeting the glutamate system with pharmacological agents may normalize the system and alleviate symptoms associated with ADHD (Carrey et al. 2007; Kavirajan 2009; Findling et al. 2007). It is worth noting that research has currently suggested that staple treatments in ADHD such as methylphenidate and amphetamines may also affect the glutamate system indirectly (Miller et al. 2013). However, here we will only discuss current pharmaceuticals approved/suggested to treat ADHD that act directly on the glutamate system, namely atomoxetine and memantine. For each drug a brief pharmacological profile will be discussed followed by research from animal models and human studies.

16.4.1 Atomoxetine: Basic Pharmacology

Atomoxetine is a non-stimulant medication associated with less abuse liability than traditional ADHD treatments (Jensen et al. 2015). Further, data suggest that it is well tolerated with minimal adverse advents in children with ADHD (Wernicke and Kratochvil 2002). Initially the primary mechanism of action of atomoxetine was thought to be as a blocker of the norepinephrine transporter (NET) with minimal activity at other neurotransmitter systems (Michelson et al. 2007a). However, more recent evidence suggests that atomoxetine also works as a non-competitive antagonist at NMDA receptors at relevant physiological levels (Ludolph et al. 2010; Miceli and Gronier 2015). In children and adolescents atomoxetine was absorbed with peak plasma concentrations occurring in 1–2 h with a half-life of approximately 3 h (Witcher et al. 2003). Generally speaking, the recommended dose of atomoxetine for children is 80 mg/day with a maximum recommended dose of 100 mg/day (Sauer et al. 2005). Atomoxetine is primarily metabolized in the liver by CYP2D6; those who are poor metabolizers use the CYP2C19 pathway (Sauer et al. 2005). Note that it is important to assess those for poor metabolism because the drug dose will need to be adjusted accordingly (Sauer et al. 2005).

16.4.2 Atomoxetine & ADHD: Evidence from Animals Models

Several studies have been conducted using atomoxetine in animal models of ADHD and impulsivity. A recent study showed that rats with a lesion in the dorsal noradrenergic ascending bundle given atomoxetine had increased performance on the 5-CSRTT task suggesting that not only can atomoxetine decrease impulsivity but that atomoxetine's effects on the NET are not the sole reason for the drug's

pharmacological efficacy (Liu et al. 2015). A separate study also found atomoxetine to decrease impulsivity as measured by the 5-CSRTT task (Ansquer et al. 2014).

Contrary to the previous studies, Dommett (2014) found that atomoxetine had no effect on SHR performance in the 5-CSRTT. The author of this study suggested that lack of statistical significance could be due to a lack of sensitivity of the test to adequately tap into the hypothetical construct of impulsivity or that the SHR is not a good model of ADHD (Dommett 2014). While these explanations cannot be completely ruled out, it is worth noting that there is ample evidence to suggest that this may not be the case; however, these topics are discussed elsewhere (Sagvolden 2000; Broos et al. 2012; Bayless et al. 2015). It is also worth noting that Dommett (2014) had a difficult time training the SHR on the 5-CSRTT with only ten out of eighteen completing the task. Thus, there may have been an issue in the way the task was set up in this experiment that may have confounded the results.

Another study showed that atomoxetine reversed locomotor activity, impaired novel object recognition, and prepulse inhibition in impulsive mice (Shibasaki et al. 2015). A study assessing probability discounting in rats showed that a low dose of atomoxetine increased choice for the larger reinforcer suggesting a decrease in impulsive choice (Montes et al. 2015). However, rats in an adjusting delay discounting procedure showed no changes in impulsivity when atomoxetine was infused into the mPFC or OFC (Yates et al. 2014). These data suggest that these two brain regions in isolation cannot account for atomoxetine's observed effects in other studies where impulsivity was shown to decrease upon drug administration.

Using DAT knockout mice another study found that atomoxetine decreased cognitive deficits on an H maze task while having no effect on hyperactivity (Del'Guidice et al. 2014). This suggests that atomoxetine may improve the cognitive symptoms seen in ADHD without having any action on the dopamine system; however, an effect on the dopamine system may be necessary to decrease symptoms of hyperactivity. Another study was also interested in assessing how atomoxetine may affect the dopamine system as well as how this drug may affect performance on an open field task (Moon et al. 2014).

In the Moon study SHR were divided into four atomoxetine treatment groups: control, 1, 5, 0.25 mg/kg/day doses (oral administration). The animals were then assessed in the open field at one-, two-, and three-week intervals and after the experiment their D2 receptor concentration from the PFC, striatum, and hypothalamus were analyzed using immunohistochemistry. It was found that the 1 mg/kg/day dose significantly decreased open field hyperactivity in the SHR and that D2 receptor concentration decreased in all brain regions in a dose-dependent manner. These data suggest that atomoxetine may decrease hyperactivity by normalizing the dopamine system (Moon et al. 2014). It is worth noting again that evidence suggests that normalizing the dopamine system may also normalize the glutamate system and vice versa, thus glutamate function may play a crucial role here as well (Miller et al. 2013).

SHR treated with atomoxetine in adolescence self-administer cocaine to a lesser degree compared to those treated with methylphenidate suggesting a decrease in potential drug abuse later in life for those taking this medication (Jordan et al. 2014,

2015). Thus, all things considered, atomoxetine may not only be superior in treating ADHD symptoms compared to traditional treatments but may also be less likely to promote abuse behaviors in the future.

16.4.3 Atomoxetine & ADHD: Evidence from Clinical Trials

Although effect sizes are usually not as large as with stimulants, several clinical trials have been conducted on atomoxetine in the ADHD population with the vast majority showing positive results (Bitter et al. 2012). For example, a six-month, placebo-controlled, double-blind trial showed an improvement in attention scores in adults with ADHD compared to placebo controls (Brown et al. 2011). In another double-blind, placebo-controlled study it was found that once-daily atomoxetine improved ADHD symptoms in adolescents according to investigator, parent, and teacher ratings (Michelson et al. 2007b). A meta-analysis conducted on 25 double-blind, placebo-controlled studies also showed that atomoxetine decreased several symptoms associated with ADHD such as hyperactivity, impulsivity, and inattention compared to placebo in children and adolescents (Schwartz and Correll 2014).

An integrated analysis of three Eli Lilly clinical studies showed that atomoxetine increased emotional control in those with ADHD and that emotional control was also found to correlate to improvements in core ADHD symptoms (Asherson et al. 2015). Note that it has also been suggested that atomoxetine is equally efficacious regardless of prior stimulant medication treatment although crossover studies are currently underway to address this question (Wehmeier et al. 2014).

In a 3-year open-label study atomoxetine was found to be more effective in adult, female ADHD patients that also had other emotional dysregulations (Marchannt et al. 2011). Further, an 8-week open-label study in Japanese adults with ADHD showed a significant improvement in ADHD symptoms and a low medication discontinuation rate (Takahashi et al. 2014). In a longer open-label study (12-weeks) atomoxetine was found to be an effective treatment in adults with ADHD that also had comorbid responsive generalized anxiety disorder (Gabriel and Violato 2011). A meta-analysis conducted on data from 13 atomoxetine studies (placebo-controlled and open-label) further concluded that this drug decreases ADHD symptoms in adolescents with little to no drug tolerance or adverse events after two years of use (Wilens et al. 2006). Overall there seems to be ample evidence that atomoxetine is safe and effective in children, adolescents, and adults with ADHD.

16.4.4 Memantine: Basic Pharmacology

Memantine is an uncompetitive NMDA antagonist with low to moderate affinity for the NMDA receptor; initially, this drug was developed for the treatment of

Alzheimer's disease (Maeng and Zarate 2007). Pharmacokinetically, memantine is absorbed readily from the gut reaching maximum plasma concentration in 3–8 h with a half-life of approximately 60–80 h (Kavirajan 2009). Most of the drug is metabolized by the kidneys with very little of the drug being metabolized in the liver by cytochrome p450 enzymes (Kornhuber et al. 2007). Memantine appears to have little side effects with the most commonly reported being dizziness, constipation, headache, hypertension, and somnolence (Sani et al. 2012). Considering increased glutamate levels are suggested in those with ADHD, it is theorized that memantine's ability to decrease glutamate signaling is a possible reason for its therapeutic value in ADHD (Miller et al. 2013).

16.4.5 Memantine & ADHD: Evidence from Animal Models

There seems to be a paucity of data assessing memantine's effects on impulsivity in animal models; however, the studies found mixed results. For example, one study showed that the time spent in the central area on a locomotor task increased in both SHR and WKY controls after a high dose of memantine (32 mg/kg) suggesting an increase in impulsivity (Sukhanov et al. 2004). However, at low memantine doses (5.6 mg/kg) the SHR group spent a smaller amount of time in the central area suggesting a decrease in impulsivity (Sukhanov et al. 2004). In this same study memantine seemed to have little to no effect on SHR and WKY rats in delay discounting suggesting that this drug did little to impulsivity (Sukhanov et al. 2004).

In another study memantine seems to increase impulsive choice in low impulsive rats (Cottone et al. 2013). However, the fact that these authors used a median split in order to divide their rats into low and high impulsive groups may confound their results; thus, this should be considered when interpreting their data. All things considered, animal models assessing the effects of memantine on impulsivity are mixed; however, clinical trials seem to present more consistent results.

16.4.6 Memantine & ADHD: Evidence from Clinical Trials

Clinical trials have assessed the efficacy of memantine for the treatment of ADHD in children, adolescents, and adults (Findling et al. 2007; Surman et al. 2013). An eight-week, open-label trial found that a dose of 10 mg/day and 20 mg/day showed dose-dependent benefits in both inattention and hyperactivity/impulsivity in adolescents with ADHD-C (Findling et al. 2007). Another open-label study showed that memantine titrated to a dose of 10 mg twice a day meliorated ADHD symptoms in adults (Surman et al. 2013).

In a randomized, double-blind, placebo-controlled clinical trial memantine in conjunction with stimulant medications was shown to improve behavior ratings on ADHD inventories in adult ADHD (Biederman et al. 2014). To the best of the

authors' knowledge, these are the few clinical trials that have assessed memantine in the ADHD population directly. However, there have been other trials assessing memantine in impulsive-like disorders such as gambling, drug addiction, and kleptomania (Biederman et al. 2014; Grant et al. 2013).

In a study by Grant et al. (2010) 28 patients diagnosed with pathological gambling received between 10 and 30 mg/day of memantine for 10 weeks. After study completion participants reported a decrease in compulsive and impulsive behaviors as well as improved cognitive flexibility (Grant et al. 2010). In other studies assessing those with substance abuse disorders it was found that memantine reduces cue-induced craving in alcoholics as well as decreases withdrawal symptoms associated with opiate abstinence (Krupitsky et al. 2007; Bisaga et al. 2001). Another study showed that a dose of 10 mg/day titrated to 30 mg/day of memantine decreased impulsive stealing behavior in those with kleptomania (Grant et al. 2013). Thus, memantine may disrupt impulsive-like behaviors associated with ADHD, gambling, and addiction. Further, memantine's action on the glutamate system is likely responsible for this therapeutic effect.

16.5 Future Perspective for Glutamate Modulating Drugs for the Treatment of ADHD

The data reviewed in this chapter presents ample evidence of glutamatergic dysfunction in ADHD. Further, data supports that using pharmaceuticals that target the glutamate system (i.e., memantine and atomoxetine) may stabilize glutamatergic signaling and meliorate the behavioral issues associated with ADHD in both animal models and humans. Another interesting thought presented in this chapter is the relatively new idea that the neurotransmitter imbalances seen in ADHD could be due to issues with how the brain obtains energy. Thus, future pharmaceuticals for the treatment of ADHD targeting the glutamate system and systems that can increase energy flow to the brain could be beneficial.

As previously mentioned, when glutamate is released from synapses and taken up by astrocytes lactate is shuttled from astrocytes to fuel neuronal firing (Wyss et al. 2011). Further, stimulation of β-adrenergic receptors on astrocytes stimulates glycogenolysis causing an increase in astrocytic glucose that is transformed to lactate and shuttled to neurons (Pellerin and Magistretti 2011). Also, stimulation of α2A-aderendergic receptors on astrocytes may increase astrocytic glycolysis in the long-term thus increasing their energy storage (Hutchinson et al. 2011). This becomes interesting considering that a current treatment for ADHD (i.e., atomoxetine) works on the norepinephrine, dopamine, and glutamate pathways and that a current hypothesis for ADHD is dopamine/norepinephrine hypo-function that in turn may cause glutamatergic hyper-function (Miller et al. 2013).

Considering the above, it is easy to imagine a situation where lack of brain energy eventually causes catecholamine hypo-function because not enough energy is

available to sustain normal neuronal firing. This catecholamine hypo-function then promotes an increase in glutamatergic firing, which then puts more energy stress on an already vulnerable system. This could create a feed-forward pathological situation that may lead to the symptoms seen in ADHD as well as other psychiatric illnesses. Thus, the development of drugs, which act similar to atomoxetine both to decrease glutamate signaling (i.e. glutamate antagonists) and increase catecholamine signaling (i.e. agonists or uptake inhibitors) may help to increase the energy supply to the brain and directly act on the neurotransmitter systems dysregulated; both effects should normalize brain physiology and meliorate the symptoms of ADHD.

As proposed by Todd and Botteron in 2001, if we consider ADHD to be primarily an issue with how the brain gets energy and that this energy problem causes neurotransmitter dysregulations then a simplistic possibility is that diet and exercise may also help to alleviate symptoms seen in ADHD. The current research from animal models suggests that the data on modulating diet to improve ADHD symptoms is mixed (Pase et al. 2015; Liso Navarro et al. 2014). However, the evidence from human studies seems promising (Heilskov Rytter et al. 2015). Although clinical anecdotes from patients with ADHD attest to the importance of exercise in reducing symptoms, evidence from animal models and human studies on exercise and ADHD are mixed but seem promising (Robinson and Bucci 2014; Piepmeier et al. 2015; Pan et al. 2015; Chuang et al. 2015). Thus, perhaps in the future a standard of care for ADHD will not only be using pharmaceuticals that work to increase brain energy but also by prescribing a certain diet and exercise regimen in order to normalize glutamate and catecholamine neurotransmission in the brain.

16.6 Conclusion

In this chapter we have reviewed current, relevant data from animal models and humans on the glutamate system and how this system may be dysregulated in ADHD. We have also alluded to how other neurotransmitter systems such as the dopamine and norepinephrine systems may interact with the glutamate system to produce ADHD symptoms. We propose that an overarching cause of all the neurotransmitter dysregulations seen in ADHD have their root in brain energetics. Current pharmacotherapies that work on the glutamate system were discussed, as were thoughts for future treatments in ADHD that work on the glutamate system either directly or indirectly. It is the hope of the authors that the data reviewed in this book chapter as well as the novel ideas discussed will be helpful to researchers currently working on treatments for ADHD and will encourage further research in this field.

Acknowledgements The authors would like to thank Hayden Scott and Luke Sparks for their help in obtaining some of the literature reviewed in this book chapter.

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Chapter 17
Glutamate, Glutamatergic Drugs and Schizophrenia

Carolina Muguruza and Luis F. Callado

Abstract Over the past two decades, increasing evidence from preclinical and human studies supports a major role of glutamatergic dysfunction in the aetiopathology of schizophrenia. Genetic, postmortem and neuroimaging studies in schizophrenia patients showed alterations in different glutamatergic elements. Thus, glutamatergic system has emerged as a promising target for the treatment of schizophrenia, especially for symptoms not addressed by current antipsychotic medications, i.e. negative symptoms and cognitive deficits. To date, several drugs aiming at restoring glutamatergic function in schizophrenia have already been researched. The main pharmacological actions of these drugs comprise (1) potentiation of the NMDA receptor function and (2) activation of metabotropic glutamate receptors (mGluRs). Different glutamatergic targets have been proposed to enhance NMDA functioning, including agonism at the glycine-binding site and inhibition of the glycine transporter (GlyT1). Additionally, agonists of the mGlu2/3 receptor and positive allosteric modulators (PAMs) of mGlu2 receptors have been tested as therapeutic bets for the treatment of schizophrenia. From all emerging glutamatergic drugs in schizophrenia, the selective GlyT1 inhibitor Bitopertin and the orthosteric mGlu2/3 receptor agonist Pomaglumetad methionil have reached Phase III clinical trials. Despite discouraging outcomes from these studies, deeper analyses on their methodological features—including patient selection, previous medications, dosages, etc.—highlight the effectiveness of these compounds in patients with schizophrenia.

Keywords Schizophrenia · Glutamate · Emerging drugs · mGlu receptors · PAMs

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_17

17.1 Schizophrenia

Schizophrenia is a common, chronic and severe psychiatric disorder that affects more than 20 million people worldwide (Charlson et al. 2018). Its onset occurs typically in late adolescence or early adulthood, and it is preceded by an initial prodromal stage and followed by psychotic exacerbations or relapses that alternate with periods of partial remissions. Schizophrenia is among the leading 20 causes of global years lived with disability (YLDs) in both males and females according to the Global Burden of Disease study 2017 (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators 2018). A meta-analysis from 50 original studies showed that, on average, only 13.5% of patients with schizophrenia met clinical and social recovery criteria (Jaaskelainen et al. 2013). Moreover, compared to general population, suicide rates among patients with schizophrenia are higher, and the mean age at death is 15 years younger (Hjorthoj et al. 2017; Chan et al. 2018). Thus, the combined social and economic costs of schizophrenia make this pathology a serious health problem worldwide with a large burden that needs attention (He et al. 2020).

The clinical features of schizophrenia can be clustered in three categories: positive symptoms, negative symptoms and cognitive deficits. Positive or psychotic symptoms include delusions (firm and fixed beliefs held in the face of contradictory evidence), hallucinations (perceptions in the absence of external stimulus that has qualities of real perceptions, most commonly experienced as hearing voices distinct from one's own thoughts) and thought disorder (disorganized behaviour and speech). Negative symptoms comprise psychomotor poverty (lack of speech, lack of spontaneous movement), social withdrawal, impairments in initiative and motivation and a reduced capacity to recognize and express emotional states. Cognitive impairments include disturbances in selective attention, working memory, executive control, episodic memory, language comprehension and social-emotional processing. Current pharmacological treatments are based on antipsychotic drugs that are effective in reducing the severity of positive symptoms (Miyamoto et al. 2012). Thanks to the advent of these drugs in the mid-1950s with the first antipsychotic chlorpromazine (Delay and Deniker 1955), many patients have been able to achieve symptomatic recovery in terms of hallucinations and delusions. However, current available antipsychotic drugs are only partially effective for negative symptoms and have no effect on cognitive impairment (Miyamoto et al. 2012). The prefrontal cortex (PFC) is the main anatomical substrate of cognitive activities, particularly working memory, and it is a cardinal component of executive functions. Neuropathological and neuroimaging studies have shown molecular and anatomical alterations of the PFC in schizophrenic patients (Lett et al. 2014). In this sense, despite psychosis being a core feature of schizophrenia, cognitive impairment precedes the onset of psychosis and constitutes a risk factor of the course of the illness (Kahn and Keefe 2013). Moreover, it has been evidenced that dysfunction in cognition and social cognition has a major impact on patients' functional status (Green 2016), representing important determinants of functional outcome for individuals with schizophrenia (Javed and Charles 2018). Thus, current treatment of

schizophrenia with the available antipsychotics rarely, if ever, produces a cure or entirely reverses symptoms of the illness (Smith et al. 2010).

Despite the efforts to elucidate the aetiopathogenic bases of schizophrenia over the last decades, the biological processes underlying this pathology remain unknown. Schizophrenia is considered a neurodevelopmental disorder with a genetic background and high heritability but also with an important environmental component (Stilo and Murray 2019; Van Os et al. 2010). Thus, schizophrenia is a multifactorial disease that reflects an interaction between genetic vulnerability and environmental contributors (Assary et al. 2018). Nevertheless, since there is a consensus that schizophrenia is a brain disorder, the putative role of neurotransmitter systems in the aetiology of the illness has been of major interest. The most widely considered neurochemical hypothesis of schizophrenia is the dopaminergic hypothesis, which goes back to the 1960s. This hypothesis is indirectly supported by two sets of findings: (1) antipsychotic drugs are dopamine receptor antagonists (Carlsson and Lindqvist 1963) and their clinical potency is strongly correlated to their ability to block dopamine D2 receptors (Creese et al. 1976; Seeman et al. 1976); and (2) drugs that increase dopamine activity, such as amphetamine, can induce psychotic symptoms in individuals who do not have schizophrenia (Angrist and Gershon 1970) and exacerbate psychotic symptoms in schizophrenic patients (Curran et al. 2004; Lieberman et al. 1987). However, the first general hyperdopaminergic hypothesis was not supported by different studies, which reported unchanged levels of dopamine metabolites in cerebrospinal fluid and postmortem brain samples in schizophrenia. Moreover, negative symptoms and cognitive deficits could not be explained by this first hypothesis. Consequently, the dopamine hypothesis was reformulated postulating that positive symptoms of schizophrenia may result from subcortical hyperdopaminergia, whereas prefrontal hypodopaminergia would be responsible for the negative symptoms and cognitive impairments (Davis et al. 1991). More recently, a "third version" of the dopamine hypothesis has been developed, which takes into consideration new evidence on genetic, neurodevelopmental, environmental and social factors linked to schizophrenia, giving rise to the "aberrant salience-hypothesis" (Howes and Kapur 2009). Despite these reformulations, the dopamine hypothesis cannot fully explain the clinical pathology and course of schizophrenia. Actually, the third version is a "dopamine hypothesis of psychosis-in-schizophrenia" rather than a hypothesis of schizophrenia (Howes and Kapur 2009).

Multiple transmitter/neural system alterations might underlie the negative symptoms and cognitive impairment of schizophrenia, which in many cases precede the onset of psychosis. During the last decades, new beyond-dopamine hypotheses of schizophrenia have emerged, contributing to a better understanding of the disease and to the discovery of novel pharmacological targets for schizophrenia treatment (Yang and Tsai 2017). Among these, the glutamatergic hypothesis of schizophrenia was one of the firsts to be formulated, pointing at the glutamatergic system as a promising avenue in the search for novel pharmacological treatments that focus especially on negative and cognitive symptoms of schizophrenia.

17.2 Glutamatergic Hypothesis of Schizophrenia: NMDA Receptor Hypofunction

Glutamate is the major excitatory neurotransmitter in the brain interacting with two types of receptors: (1) the ionotropic receptors with NMDA, Kainate, and AMPA receptor subtypes connected to or representing ion channels, and (2) the metabotropic glutamate receptors (mGluRs), comprising groups I to III with a total of eight identified subtypes, which activate G-protein coupled signal transduction (Nakanishi 1992). NMDA receptors are widely expressed throughout the brain cortex and play a critical role in functions that have been proved to be altered in patients with schizophrenia, such as synaptic plasticity, learning and memory formation.

The hypothesis of an NMDA receptor hypofunction in schizophrenia arises from the observation that the administration of NMDA receptor non-competitive antagonists, such as phencyclidine (PCP) and the dissociative anaesthetic ketamine, was able to induce schizophrenia-like symptoms in healthy individuals (Javitt and Zukin 1991; Olney and Farber 1995; Stone et al. 2008). Moreover, these drugs reproduce not only the positive/psychotic symptoms of the disease—induced also by dopamine-increasing drugs like amphetamine—but also other core symptoms of schizophrenia such as cognitive impairments and negative symptoms (Krystal et al. 2005). In this sense, the administration of NMDA receptor antagonists including PCP, ketamine and dizocilpine (MK-801), has been widely used as a preclinical model of schizophrenia (Bondi et al. 2012). In addition, another finding that supports the NMDA receptor hypofunction hypothesis of schizophrenia is the existence of an anti-NMDA receptor encephalitis, which is an autoimmune disorder with schizophrenia-like symptoms (Dalmau et al. 2007). This disorder is caused by the binding of pathogenic NMDA receptor autoantibodies to the extracellular domain of the receptor inducing its blockage and internalization and producing neuronal dysfunction (Hughes et al. 2010).

Nevertheless, the precise downstream molecular mechanisms by which NMDA receptor antagonists induce schizophrenia-like symptoms have not been fully elucidated. Since NMDA receptors are located on brain circuits that regulate dopamine release, it has been suggested that cortical dopaminergic deficits in schizophrenia may also be secondary to underlying glutamatergic dysfunction (Javitt 2010). However, and paradoxically, the antagonism of NMDA receptors by PCP or ketamine induces an overall profound cortical activation and even neurotoxicity in both humans and rodents (Breier et al. 1997; Ellison 1995; Suzuki et al. 2002; Vollenweider et al. 1997). This controversy has been explained as a consequence of a preferential action of these antagonists on NMDA receptors located in GABAergic interneurons, suggesting that a disinhibition of pyramidal neurons causes the cortical excitation (Homayoun and Moghaddam 2007). There are further theories that try to explain the preferential inhibition of NMDA receptors located in GABAergic interneurons, which include NMDA antagonism-induced changes in reactive oxygen species as a main mechanism (Behrens et al. 2007). An aberrant

GABA interneuron activity leading to disruption of the excitatory/inhibitory cortical balance has also been proposed as a core pathophysiological mechanism underlying cognitive dysfunction in schizophrenia (Dienel and Lewis 2019). Thus, a specific hypofunction of NMDA receptors located in cortical GABAergic interneurons might explain the cortical dysfunction in schizophrenia. If this is the case, there are a few questions that remain unanswered, such as which is the cause of NMDAR dysfunction and what approaches may be most effective to restore the underlying alterations (Moghaddam and Javitt 2012).

In addition to the evidence related to NMDA receptor antagonism, postmortem, genetic and neuroimaging studies have found that several components of glutamatergic signaling system are altered in schizophrenic patients (see Sect. 17.3). All these indirect findings support a glutamatergic hypothesis of schizophrenia, and consequently, the search of novel drugs that could restore the glutamatergic deregulation in the disease and provide a therapeutic alternative, especially for the symptoms not addressed by current antipsychotic drugs. In this sense, numerous preclinical and clinical studies have elucidated several potential targets to increase NMDA receptor function and equilibrate glutamatergic tone, including NMDA receptor co-agonists, inhibitors of the glycine transporter and agonists and positive allosteric modulators (PAMs) of mGluRs (see Sect. 17.4).

17.3 Evidence of Altered Glutamatergic System in Schizophrenia

17.3.1 Genetic Studies

Research on genetic alterations related to schizophrenia is a valuable approach that can contribute to understand the cause of the disease (Harrison and Weinberger 2005). Genetic findings support the role of the glutamatergic system in schizophrenia pathophysiology. A genome-wide association study (GWAS), with over 100,000 controls and almost 40,000 schizophrenia cases, found 108 conservatively defined loci associated with schizophrenia. These loci met genome-wide significance for several genes that are involved in glutamatergic neurotransmission (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Examples of the glutamatergic elements encoded by these genes associated with schizophrenia include the mGlu3R (GRM3), the NR2 subunit of NMDA receptor (GRIN2A), the serine racemase (SRR) and the AR1 subunit of AMPA receptor (GRIA1) (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Moreover, studies on large copy-number variants (CNVs) and single-nucleotide variants—exerting larger effects than common single-nucleotide polymorphisms (SNPs)—have also found genes related to glutamatergic elements encoded in the variants associated with schizophrenia. Thus, NMDAR network genes have proved to be highly enriched in CNVs overall, primarily in duplications (Pocklington et al. 2015).

Concerning Group I mGluRs, deleterious nonsynonymous SNPs have been found in the gene encoding the mGlu1R (GRM1) in schizophrenia (Ayoub et al. 2012; Frank et al. 2011b). In the gene encoding the mGlu5R (GRM5), two independent variants—rs60954128 and rs3824927—have been associated with cognitive impairments and right hippocampal volume reduction in schizophrenia (Matosin et al. 2018). It has been also reported that the functional polymorphisms (rs4354668 and rs2731880) of the glutamate transporters EAAT1 and EAAT2—which are associated with lower transporter expression and higher glutamate levels— are associated with a poorer cognitive performance in patients with schizophrenia (Spangaro et al. 2014).

Genetic alterations of the genes encoding the Group II mGluRs (GRM2 and GRM3) have also been investigated in schizophrenia. For the GRM2, negative results have been reported in linkage studies of the region encoding this gene (Moreno et al. 2009) and no associations with schizophrenia were found in studies searching for candidate polymorphisms in GRM2 alleles (Joo et al. 2001). However, associations between SNPs in the GRM3 and schizophrenia have been consistently reported (Chen et al. 2005; Cherlyn et al. 2010; Egan et al. 2004; Fujii et al. 2003; Sartorius et al. 2008; Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014), although these findings have not been replicated in some population-based genetic studies (Marti et al. 2002; Tochigi et al. 2006). The vast majority of the polymorphisms of GRM3 associated with schizophrenia are located in non-coding regions, making it difficult to elucidate the pathophysiological role of these genetic alterations in schizophrenia. In this regard, Egan et al. (2004) proposed that the GRM3 genotype could alter the glutamatergic transmission through a mechanism that leads to an increase in the risk for schizophrenia. Thus, on the one hand, GRM3 intronic variation (hCV11245618) carriers showed poorer performance on several cognitive tests and, on the other hand, the GRM3 variant was found to be associated with lower mRNA levels of EAAT2 glutamate transporter in postmortem brain of schizophrenic subjects (Egan et al. 2004). In this context, it has been suggested that GRM3 genotype affects the risk to develop schizophrenia by means of altered EAAT2 expression and glutamate neurotransmission (Egan et al. 2004).

17.3.2 Postmortem Brain Studies

Different elements of the glutamatergic system have been researched in the postmortem brain of schizophrenic subjects, ranging from the structure of glutamatergic neurons to receptors and glutamate transporters (Hu et al. 2015). These studies reached variable outcomes depending on the precise glutamatergic element and the brain region under study. Many studies focused on cortical regions, as it is the main anatomical substrate of the cognitive impairment associated with schizophrenia and where dysfunction in glutamatergic signaling might have a major role (Coyle 2017; Thomas et al. 2017). The main outcomes related to mRNA and protein expression of

major ionotropic receptors, glutamate transporters as well as metabotropic glutamate receptors in the schizophrenia postmortem brain are summarized below.

17.3.2.1 Ionotropic Glutamate Receptors

The mRNA and protein expression of the different subunits conforming the NMDA, AMPA and Kainate receptors have been addressed in schizophrenia postmortem brain studies. Overall, decreased or unchanged cortical expression has been reported for the different subunits of the NMDA receptor, but increases have also been found (Hu et al. 2015). Regarding the obligatory NR1 subunit of the NMDA receptor, a quantitative meta-analysis including 12 postmortem studies reported a significant decrease in mRNA and protein expression in the PFC of schizophrenic patients, when compared to controls (Catts et al. 2016). Studies focusing on the variable subunits of the NMDA receptor (NR2-A, B, C and D and NR3A) reached controversial outcomes (Hu et al. 2015). In this sense, it is important to notice that multiple receptor isoforms with distinct brain distributions and functional properties arise by selective splicing of the NR1 transcripts (Zukin and Bennett 1995) and differential expression of the NR2 subunits (Paoletti et al. 2013). This structural and functional heterogeneity hinders the interpretation of subunit individual outcomes. In this regard, a significantly increased expression of NR1 C2' splicing variant has been reported in the anterior cingulate cortex in schizophrenia—without changes in the PFC—which suggests an altered cell processing of the NMDA receptor in this cortical region (Kristiansen et al. 2006). A recent study found a significant reduction in NR1 isoforms containing C1 splicing variant in the PFC of schizophrenic subjects when compared to controls, but no changes were found in total NR1 protein levels (Rodriguez-Munoz et al. 2017). Since the NR1 C1 subunit assists NMDARs in the formation of stable complexes with GPCRs, this alteration in the composition of NR1 subunits leads to a reduction in GPCR-NMDAR cross-regulation in schizophrenia (Rodriguez-Munoz et al. 2017). In addition to the individual subunits, the whole NMDA postsynaptic density (PSD) has also been investigated in schizophrenia. Strikingly, total NMDA receptor in PSD was found increased in schizophrenia (Banerjee et al. 2015) even though decreased NR1 subunit levels (Catts et al. 2015) and NR2 subunit hypofunction (Banerjee et al. 2015) have been reported.

Fewer studies have been conducted on Kainate and AMPA receptors in postmortem brain of schizophrenic subjects. Several works have measured the mRNA expression of different subunits of AMPA and Kainate receptors in the cortex of schizophrenic subjects with inconsistent results (Hu et al. 2015). To date, only two studies have evaluated the protein expression of AMPA receptor in the cortex of schizophrenic subjects, and they have found a decrease in the four subunits of the receptor (Corti et al. 2011; MacDonald et al. 2015). This decreased protein expression of AMPA receptor subunits in cortical regions is in line with the hypothesis of a reduced AMPA receptor activity in schizophrenia contributing to a pathological modulation of NMDA receptors (Huganir and Nicoll 2013). However, previous binding studies found either no change or slightly increased AMPA receptor binding

in the PFC in schizophrenia (Healy et al. 1998, Kurumaji et al. 1992). These contradictory outcomes could be a consequence of the different pool of receptors targeted by the different technical approaches (radioligand binding vs. immunodetection) used to assess the receptor levels.

17.3.2.2 Glutamate Transporters

Astrocytic EAAT1 and EAAT2, the primary glutamate transporters in the human CNS in charge of glutamate reuptake from the synaptic cleft, have been investigated in schizophrenia. Since cortical glutamate excitotoxicity has been associated with schizophrenia, a dysregulation of EAATs may be involved in the resulting neuropathology (O'Donovan et al. 2017; Parkin et al. 2018). Increased levels of EAAT1 mRNA have been reported in the cortex of subjects with schizophrenia (Bauer et al. 2008; Scarr et al. 2018). However, no changes in mRNA expression have also been reported depending on the cortical area under examination (Bauer et al. 2008; Lauriat et al. 2006). Regarding the protein expression of EAAT1 in schizophrenia, monomeric EAAT1 was found decreased in the dorsolateral PFC (DLPFC) of elderly subjects with schizophrenia (Bauer et al. 2008). Decreased (anterior cingulate cortex) or unchanged (DLPFC) glycosylation of the transporter has also been shown (Bauer et al. 2010). Both mRNA and protein expression of EAAT2, which accounts for the 90% of glutamate clearance (Suchak et al. 2003), have been shown unchanged in the cortex of schizophrenic subjects (Bauer et al. 2008). However, glycosylation of EAAT2 was found reduced in the brains of schizophrenic subjects (Bauer et al. 2010). Together, these findings indicate that EAAT1/2 may be involved in schizophrenia pathogenesis (Parkin et al. 2018).

17.3.2.3 Metabotropic Glutamate Receptors

Alterations in both, mRNA and protein expression of different mGluRs have been reported in schizophrenia postmortem brain. Regarding Group I mGluRs, more research has been focused on the mGlu5R than on mGlu1R subtype in schizophrenia; probably due to the major regulating role of mGlu5Rs over NMDAR function (Matosin et al. 2017). One study has shown higher mRNA levels of mGlu1αR in the DLPFC of schizophrenic subjects when compared to controls (Volk et al. 2010). This altered mRNA expression is consistent with the results reporting increased protein expression of mGlu1αR in the prefrontal cortex of schizophrenic subjects (Gupta et al. 2005). This study also evidenced no changes in mGlu5R protein expression in the PFC of schizophrenic subjects (Gupta et al. 2005). In the same way, most of the studies assessing either mRNA or protein expression of mGlu5R in the postmortem brain of schizophrenic subjects have reported no alterations when compared to controls, regardless of the brain area under examination (Matosin et al. 2017). However, increased protein expression without changes in mRNA levels of mGlu5R has been shown in the same PFC brain samples (Brodmann area 46) from

schizophrenic subjects compared to controls. This suggests that the rate of mGlu5R protein synthesis or degradation might be affected in schizophrenia depending on the examined brain region (Matosin et al. 2015). In terms of functional assessment of mGlu5R in schizophrenia, a reciprocal interplay between NMDA and mGlu5R pathways has been suggested. Thus, a reduction in mGlu5R signaling accompanied by a reduced GNR2 phosphorylation has been reported in the DLPFC of schizophrenic subjects (Wang et al. 2018). The authors state that the decrease in NR2 phosphorylation can be precipitated by mGlu5R hypofunction and that increased mGlu5R phosphorylation can result from decreased NMDAR function (Wang et al. 2018). Overall, the data reported to date regarding mGlu5R alterations in postmortem brain of schizophrenic patients are inconclusive. It seems that mGlu5R could play a significant role in the pathophysiology of schizophrenia. Nevertheless, further research is needed to understand how mGlu5R might be involved in the neurobiology of this disorder and especially to support the progression of mGlu5R-targeting drugs into the clinic for schizophrenia treatment (Matosin et al. 2017).

The mRNA and protein expression of Group II mGluRs, comprising mGlu2R and mGlu3R receptors, has been assessed in the postmortem brain of schizophrenic subjects by means of different approaches. Overall, PFC levels of mGlu3R mRNA have been shown unaltered in schizophrenia (Egan et al. 2004; Ghose et al. 2008; Gonzalez-Maeso et al. 2008; Ohnuma et al. 1998). In contrast, different outcomes have been reported related to mGlu2R mRNA expression in PFC schizophrenia when compared to controls, including increases (Ghose et al. 2008) and decreases (Gonzalez-Maeso et al. 2008).

Regarding protein expression of Group II mGluRs, the differentiation of the subtypes 2 and 3 has been a problematic issue, due to the high homology between these proteins and the lack of selective antibodies for each subtype. Thus, early studies using non-specific antibodies achieved different outcomes in the PFC of schizophrenic subjects when compared to controls, including unaltered (Crook et al. 2002; Gupta et al. 2005) and increased (Gupta et al. 2005) mGlu2/3R expression, also depending on the Brodmann area assessed. Posterior studies have evaluated selectively the immunoreactivity of the mGlu3R in schizophrenia, and they have also reported non-concordant results (Corti et al. 2007; Garcia-Bea et al. 2016; Ghose et al. 2009). Only one study has evaluated the immunoreactivity of mGlu2R in schizophrenia PFC showing no alterations when compared to controls (Dean et al. 2019). The receptor density of mGlu2/3R has also been evaluated in the PFC of schizophrenic subjects by means of radioligand binding techniques. These studies are limited by the use of non-selective radioligands of receptor subtypes. In this sense, decreased (Gonzalez-Maeso et al. 2008) or unchanged (Frank et al. 2011a; McOmish et al. 2016) mGlu2/3R density has been reported in the PFC of schizophrenic subjects when compared to controls.

In addition to protein expression and receptor density, one study has evaluated the functional signaling of mGlu2/3R in postmortem PFC of schizophrenic subjects. The authors showed that the activation of Gq/11 signaling by the mGlu2/3 agonist LY379268 was significantly decreased in schizophrenic subjects when compared to controls and no differences were found in the Gi/o LY379268-dependent activation

(Moreno et al. 2016). This investigation on the G-protein signaling pattern in schizophrenia was carried out based on previous data showing that mGlu2/3R agonist signaling requires expression of 5-HT2A and mGlu2 heteromers (Moreno et al. 2016). The implications of the organization of mGlu2 and 5-HT_{2A} receptors into heteromers on (1) the pattern of G-protein coupling and (2) the antipsychotic effects of mGlu2 activation is discussed below (Sect. 17.5).

Overall, due to the inconclusive findings from postmortem studies, the status of Group II mGluRs in schizophrenia remains unclear. Further investigation of the level of expression and function of mGlu2R and mGlu3R in postmortem human brain of schizophrenic subjects and controls is needed (Muguruza et al. 2016).

17.3.3 In Vivo Neuroimaging Studies

Glutamate function has been investigated in vivo in schizophrenic patients by means of neuroimaging techniques including proton magnetic resonance spectroscopy (1H-MRS), positron-emission tomography (PET) and single-photon emission computed tomography (SPECT) (Li et al. 2019; Poels et al. 2014b). 1H-MRS has been used to measure the levels of glutamate (Glu), glutamine (Gln) and the combination of both (Glx) as markers of glutamatergic functioning. In healthy individuals, 1H-MRS studies reported that acute administration of ketamine is able to increase glutamine levels in the anterior cingulate cortex (Rowland et al. 2005; Stone et al. 2012). 1H-MRS studies have been conducted in patients with first-episode psychosis and chronic schizophrenia and compared to results on healthy controls (Poels et al. 2014a). Concerning cortical regions, when compared to controls, elevated glutamatergic levels have been evidenced in antipsychotic-naïve/free schizophrenic patients (Poels et al. 2014a; Salavati et al. 2015). However, no changes in glutamate-related metabolites have been reported in a recent systemic review and meta-analysis of 1H-MRS studies on antipsychotic-naïve/free patients with schizophrenia (Iwata et al. 2018). Despite not all studies being in accordance, the majority of the results revealed similar glutamatergic levels of medicated patients and healthy controls in the PFC, suggesting that antipsychotic treatment may decrease glutamatergic levels (Poels et al. 2014a). The outcomes from studies that performed longitudinal measurements in patients in unmedicated and medicated states do not consistently support these potential effect of antipsychotic treatment on glutamatergic indices (Aoyama et al. 2011; Bustillo et al. 2010; Szulc et al. 2005; Theberge et al. 2007;). A meta-analysis including studies that examine longitudinal changes in brain glutamate metabolites in patients with schizophrenia before and after initiation of first antipsychotic treatment or a switch in antipsychotic treatment reported a reduction in brain glutamate metabolites with antipsychotic treatment (Egerton et al. 2017). Nevertheless, further investigation is needed to confirm the impact of antipsychotic medication in cortical glutamatergic levels in schizophrenic patients (Egerton et al. 2017). Additionally, there is considerable interest in the potential use of 1H-MRS to predict treatment response in schizophrenia, holding the theory that poor responders

to conventional dopaminergic antipsychotic treatments may have more glutamatergic basis to their illness (Howes et al. 2015). A meta-analysis of 1H-MRS studies in schizophrenia reported significant elevations in Glu and Glx in the basal ganglia, Gln in the thalamus and Glx in the medial temporal lobe, but no significant alterations were found for neither Glu, Gln nor Glx in the medial frontal cortex of schizophrenic patients (Merritt et al. 2016). Despite the lack of associations in cortical regions, this study showed that schizophrenia is associated with elevations in glutamate-related metabolites across several brain regions consistent with the hypothesis that there is excess glutamatergic neurotransmission in this condition (Merritt et al. 2016). In terms of clinical correlations with glutamatergic levels in medial and dorsolateral PFC of schizophrenic patients, results are either negative or inconsistent (Poels et al. 2014a). Interestingly, a recent study reported higher Glx levels in the dorsolateral PFC of patients with lifetime auditory verbal hallucinations (AVH) as compared to patients without lifetime AVH, suggesting a mediating role for Glx in AVH (Curcic-Blake et al. 2017).

Taking into account the limitations of the measurements obtained by means of 1H-MRS, including the inability to distinguish between intracellular or extracellular compartments, or between intra- or extra-neuronal compartments, PET and SPECT techniques provide a more selective measurement of brain neurochemistry than 1H-MRS (Poels et al. 2014b).

PET and SPECT studies in healthy subjects showed an impact of NMDA blockade (using ketamine) on dopaminergic indices, providing initial support for the glutamate/NMDA hypothesis of schizophrenia. The following development of PET and SPECT imaging of the glutamate system allowed the direct in vivo measurements of glutamatergic indices, which are necessary to translate preclinical and clinical findings into effective therapies (Poels et al. 2014b). Despite the rapid advances in preclinical characterization of PET/SPECT ligands targeting different glutamatergic elements, to date only a few clinical studies have been conducted to assess direct measurements of glutamatergic elements in vivo. SPECT studies in healthy volunteers showed that ketamine infusion led to decreases in NMDAR availability. Moreover, studies on individuals with schizophrenia reported decreased NMDAR availability in the hippocampus of drug-free patients. Together, neuroimaging studies provide support for the glutamate hypofunction hypothesis of schizophrenia and encourage further development of glutamatergic-based treatments that increase the activity of NMDAR (Poels et al. 2014b).

17.4 Glutamatergic Elements as Drug Targets for Schizophrenia and Emerging Glutamate Modulating Drugs

As detailed above, genetic, postmortem and neuroimaging studies, together with the broad preclinical investigations in the field, evidence that glutamatergic dysfunction plays a crucial role in schizophrenia. Therefore, the development of compounds

aimed at modulating glutamatergic functions for schizophrenia treatment has been of great interest in the last decade (Hashimoto et al. 2013; Li et al. 2019; Nicoletti et al. 2019; Noetzel et al. 2012; Stansley and Conn 2018; Zink and Correll 2015).

Two main pharmacological strategies have been proposed to restore the altered glutamatergic function in schizophrenia: (1) the potentiation of NMDA receptor function through the modulation of different glutamatergic targets, i.e. the NMDA receptor Glycine Binding Site, the glycine transporter and AMPA receptors and (2) the activation of mGluRs with orthosteric agonists or positive allosteric modulators (PAMs). A schematic drawing of the glutamatergic elements investigated as potential pharmacological targets for schizophrenia treatment is presented in Fig. 17.1. A summary of the outcomes regarding the most promising glutamatergic compounds that reached assessment for schizophrenia treatment in clinical trials is presented below.

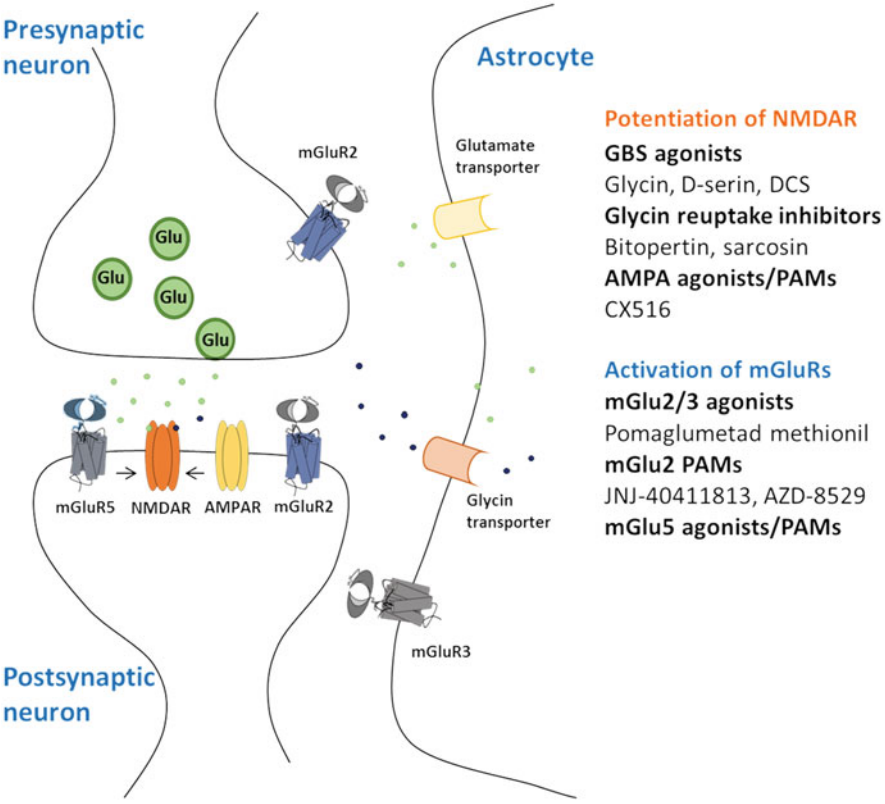

Fig. 17.1 Schematic representation of the glutamatergic pharmacological targets investigated for schizophrenia, including the glutamatergic compounds that reached phase II or III in clinical trials. (*DCS* D-cycloserine, *Glu* glutamate, *PAM* positive allosteric modulator)

17.4.1 Potentiation of NMDA Receptors

The accumulating evidence supporting the NMDA receptor hypofunction hypothesis in schizophrenia has driven the development of drugs targeting this receptor. Due to the potential neurotoxicity of direct agonists at the NMDA receptor (Lynch and Guttmann 2002), one of the main approaches to potentiate NMDA receptor function focused on targeting the glycine-binding site (GBS) of the receptor (Peyrovian et al. 2019). As both, glutamate and glycine binding are required for the activation of the NMDA receptor, agonists at the GBS have been investigated for pharmacological development in schizophrenia in order to potentiate NMDA receptor neurotransmission. Clinical trials have been conducted to evaluate the impact of the GBS agonists Glycine, D-Serine and D-cycloserine (DCS) on positive, negative and cognitive symptoms in schizophrenia (Zink and Correll 2015). Glycine is an obligatory co-agonist at the GBS of the NMDA receptor and it is required for NMDA complex activation by glutamate. Several small trials found positive efficacy for glycine as adjunctive therapy for schizophrenia (Zhand et al. 2019). However, the only available large multicentre study on the use of add-on glycine did not replicate the positive results (Buchanan et al. 2007). Two meta-analysis concluded that, as an adjuvant to non-clozapine antipsychotics, glycine improves multiple symptom domains; however, it worsens them when added to clozapine (Singh and Singh 2011; Tsai and Lin 2010).

D-serine is an endogenous selective full agonist at the GBS of the NMDA receptor. Clinical studies have been conducted in schizophrenia patients to evaluate the use of D-serine alone or in combination with antipsychotics to determine its effectiveness as a therapeutic agent (MacKay et al. 2019; Zhand et al. 2019). Positive as well as negative clinical findings have been reported for D-Serine administered alone or in combination with usual antipsychotics. In this sense, inconsistent results for the therapeutic benefit of D-serine to improve the negative and cognitive symptoms of the illness have been evidenced in clinical trials using low doses. However, more consistent improvements have been found at doses of 60 mg/kg/d or higher (MacKay et al. 2019). In summary, D-serine may be useful as adjuvant treatment for schizophrenia (Singh and Singh 2011; Tsai and Lin 2010), but due to the high doses required, more studies at longer time intervals should be conducted to ensure patient safety (MacKay et al. 2019).

DCS acts as a partial GBS agonist at NMDA receptor at lower doses, but at high doses it can act as a functional antagonist. It is the most studied NMDA receptor co-agonist in clinical trials for schizophrenia treatment, with 19 clinical trials to date (Zhand et al. 2019). Despite positive outcomes of DCS in the improvement of negative symptoms in schizophrenia in initial trials, this result was not replicated in several subsequent studies (Goff 2015). Moreover, meta-analysis results showed that DCS did not improve any symptom domain in clinical trials with schizophrenic patients (Tsai and Lin 2010). Another meta-analysis on the effects of glutamate positive modulators focusing on cognitive deficits in schizophrenia showed no differences between glycine, D-serine and DCS compared to placebo in terms of

overall cognitive function (Iwata et al. 2015). Given the poor CNS bioavailability of glycine and D-serine and the lack of full agonist activity by DCS, it remains unclear whether the GBS target was adequately tested in clinical trials (Goff 2015). Thus, further research is needed to elucidate the optimal dose ranges and route of administration of these drugs acting on the GBS of NMDA receptors.

In addition to acting directly on the GBS, the increase of glycine levels by means of inhibition of the glycine transporter (GlyT1) has been considered as a therapeutic approach for schizophrenia treatment. In this sense, two glycine transporter inhibitors have been tested in clinical trials: bitopertin, a selective and potent GlyT1 inhibitor; and sarcosine, a potent endogenous non-selective GlyT1 inhibitor that also acts as an NMDA receptor GBS co-agonist. Bitopertin, which was created and developed by the pharmaceutical company F. Hoffmann-La Roche (Pinard et al. 2018), is, to date, the most advanced GlyT1 inhibitor in clinical assessment and the only one that completed phase III clinical studies as an adjunctive treatment for schizophrenia (Bugarski-Kirola et al. 2016, 2017). Despite the successful results in phase II studies achieved in 2010 in patients with schizophrenia, overall negative phase III data was obtained in 6 multicentre studies carried out in outpatient clinics in Asia, Europe and North and South America (Bugarski-Kirola et al. 2016, 2017). Only one study, NightLyte, met the primary endpoint where the Positive and Negative Syndrome Scale Positive Symptom Factor Score significantly differed from placebo at week 12, and only at the 10-mg dose (Bugarski-Kirola et al. 2016). The findings from these studies raise important questions at technical, conceptual and regulatory levels that might have affected the ability to detect significant treatment effects (Javitt 2016). These issues include: (1) high placebo response, (2) inclusion of individuals receiving several simultaneous antipsychotic medications and (3) strong inverted U-shaped dose–response curve, leading to a very narrow therapeutic window (Javitt 2016).

Outcomes from phase II clinical trials with sarcosine as adjunctive treatment have shown consistent and highly significant beneficial effects in a range of clinical domains as shown in two independent meta-analyses (Singh and Singh 2011; Tsai and Lin 2010). However, as for other NMDA receptor modulators (glycine, D-serine and DCS), no therapeutic potential was demonstrated when added to clozapine (Singh and Singh 2011; Tsai and Lin 2010). Promising results have been reported for sarcosine, but further large-scale trials are required to replicate current findings. As has happened with other agents such as bitopertin, success in small-scale studies does not predict positive effects in larger scale studies. Moreover, a meta-analysis focusing on the effects of glutamate positive modulators (including sarcosine) on cognitive deficits in schizophrenia found that, as a group and individually, the compounds were not superior to placebo in terms of overall cognitive function (Iwata et al. 2015). The authors conclude that glutamate positive modulators may not be effective in reversing overall cognitive impairments in patients with schizophrenia as adjunctive therapies (Iwata et al. 2015).

A recent randomized, double-blind, placebo-controlled trial evidenced that adjunctive treatment with sarcosine plus benzoate (a D-amino acid oxidase (DAAO) inhibitor that prevents D-serine degradation), but not sarcosine alone,

improved the cognitive and global functioning of patients with schizophrenia (Lin et al. 2017). The authors conclude that a combination of NMDA-enhancing agents (sarcosine and benzoate), but not sarcosine alone, can improve cognitive function in patients with chronic schizophrenia (Lin et al. 2017). Nevertheless, future larger sized studies are needed to corroborate this combination benefits.

Activation of AMPA receptors has also been proposed as an approach to enhance NMDA receptor function in schizophrenia. In addition to the occupancy by two neurotransmitters (glutamate and an agonist at the GBS) the NMDA receptor activation requires the depolarization of the cell via AMPA gated channels. Since full agonist at AMPA receptors has a low seizure threshold and is poorly tolerated, positive allosteric modulators (PAMs) have been proposed as potential therapies for schizophrenia (Ward et al. 2015). Two clinical studies using adjunct treatment with the low-potency AMPA PAM (Ampakine) CX516 in schizophrenic patients have shown inconsistent results. In the first trial, improved attention and memory were evidenced in patients treated with CX516 as adjuvant treatment of clozapine (Goff et al. 2001). Nevertheless, this finding was not reproduced in an add-on trial of this compound with risperidone, olanzapine or clozapine (Goff et al. 2008). A posterior clinical study (NCT00425815) testing Org 24,448 (CX691, another ampakine) for cognitive deficits in schizophrenia as adjuvant treatment to non-clozapine antipsychotics was withdrawn (terminated at Sponsor's request) (Li et al. 2019). Trials of more potent AMPA PAMs in schizophrenia have not been reported.

17.4.2 Activation of mGlu Receptors

Metabotropic glutamate receptors (mGluRs) can modulate glutamatergic tone and its phasic release, refining the activity of ionotropic glutamate receptors in a subtle manner. These characteristics have placed mGluRs in the spotlight as promising targets for the development of drugs that restore glutamatergic function in schizophrenia (Muguruza et al. 2016; Nicoletti et al. 2019; Stansley and Conn 2018; Vinson and Conn 2012).

Among the different subtypes of receptors comprising the mGluRs, the Group I mGlu5R subtype has gained attention in the last years as a potential target to treat schizophrenia, especially regarding the cognitive symptoms associated with the disease (Matosin et al. 2017). Metabotropic mGlu5R are colocalized with NMDA receptors, they share the same scaffolding proteins and have the ability to mediate postsynaptic NMDA receptor currents (Attucci et al. 2001; Mannaioni et al. 2001) facilitating NMDA-induced long-term potentiation. Thus, the modulation of mGlu5Rs has been proposed as an alternative pathway to selectively modulate the NMDA receptors. Preclinical studies with mGlu5R PAMs in schizophrenia models have shown the ability to reverse the positive, negative and cognitive schizophrenia-like symptoms in rodents (Gastambide et al. 2012; Matosin and Newell 2013; Newell 2013). However, excitotoxicity may be a limitation of mGlu5R PAMs to overcome clinical studies, since preclinical data showed that these agents can exert

neurotoxic effects (Parmentier-Batteur et al. 2014). Despite the activation of mGlu5Rs being a new target for glutamatergic restoring in schizophrenia, the mechanistic basis of their activation also involves the potentiation of NMDA currents. Since the mGlu5R PAMs exert a final action similar to that exerted by NMDAR enhancers (Sect. 17.4.1.), it is not surprising that these drugs induce similar excitotoxic effects. These adverse effects of mGlu5R PAMs must be explored and resolved before jumping into clinical research. In this context, there is much to consider regarding the viability of mGlu5R as a novel therapeutic target for schizophrenia, as well as the implementation of its PAMs, including investigation of mGlu5R structure, signaling bias and other pathological evidence in schizophrenia (Matosin et al. 2017).

The group II mGluRs is comprised by the high homology subtypes mGlu2 and mGlu3 receptors. These receptors are mainly expressed presynaptically in cortical pyramidal neurons and coupled to Gi/o inhibitory proteins. An excess of glutamatergic activity leads to the activation of these receptors, which, by means of a negative feedback mechanism, inhibit the release of glutamate. As stated above, an increased cortical synaptic activity of glutamate due to disinhibition of pyramidal neurons—as a consequence of reduced NMDAR activity on inhibitory interneurons—has been proposed in schizophrenia. Based on this mechanism, normalizing excess glutamate levels by mGlu2/3R agonists and PAMs has been proposed and tested as a therapeutic mechanism for schizophrenia treatment in preclinical and clinical studies (Ellaithy et al. 2015; Muguruza et al. 2016).

The oral prodrug of the orthosteric mGlu2/3R agonist LY404039, Pomaglumetad methionil (LY2140023) developed by Eli Lilly and Company, became the first non-monoaminergic agent with similar efficacy to conventional antipsychotic drugs for the treatment of positive and negative symptoms of schizophrenia in a phase II clinical trial (Patil et al. 2007). Unfortunately, further trials reached unsuccessful outcomes. One follow-up multicentre phase II trial was inconclusive, with a high placebo response rate (Kinon et al. 2011), and another phase II open-label study found that pomaglumetad was inferior to a comparison atypical antipsychotic (Downing et al. 2014). Finally, another study found no benefit of adjunctive treatment with pomaglumetad versus placebo for negative symptoms in patients with schizophrenia receiving treatment with atypical antipsychotics (Stauffer et al. 2013). In August 2012, Eli Lilly decided to stop phase III trials as one of the trials, closest to completion, failed to meet its primary endpoint. An exploratory reanalysis study with the clinical data on pomaglumetad treatment defined two patients subpopulations based upon medication exposure during the 2 years before study entry (Kinon et al. 2015). This analysis has demonstrated that, when treated with pomaglumetad, only patients early-in-disease or previously treated with antipsychotics with prominent dopamine D2 receptor antagonist activity exhibited significantly greater improvement when compared to those receiving placebo (Kinon et al. 2015). Conversely, patients previously treated with atypical antipsychotics (with prominent serotonin 5-HT2A receptor antagonist activity) evidenced no greater response than placebo (Kinon et al. 2015). Kinon et al. conclude that the demonstration of antipsychotic efficacy of a potential glutamate-based pharmacotherapy for

schizophrenia may require the identification of appropriate patient subgroups, since the treatment responsiveness may be fundamentally related to dysregulation of central nervous system glutamatergic tone. The implications of targeting serotonin 5-HT2A receptors (5-HT2ARs) chronically with atypical antipsychotic treatments on mGlu2R agonists effectiveness are discussed in Sect. 17.5. Currently, there is an active clinical trial with pomaglumetad entitled "Glutamate Reducing Interventions in Schizophrenia" recruiting patients at clinical high risk for psychosis to determine the potential reductions of glutamate and metabolism (ClinicalTrials.gov registry number: NCT03321617).

Positive allosteric modulators of mGlu2R have also reached evaluation in clinical trials for schizophrenia treatment. Potential advantages of these compounds in comparison with the mGlu2/3 receptor orthosteric agonists might include subtype selectivity, a better central nervous system penetration and avoidance of receptor desensitization (Trabanco et al. 2019). To date, two mGlu2R PAMs have entered clinical trials: JNJ-40411813 (also known as ADX71149) from Janssen Pharmaceuticals, Inc. and Addex Therapeutics, and AZD8529 from AstraZeneca. JNJ-40411813 demonstrated efficacy in patients with residual negative symptoms in a phase II clinical trial (Hopkins 2013). However, both compounds, JNJ-40411813 and AZD8529, failed to show robust efficacy in proof of concept and phase IIa studies in schizophrenia (Litman et al. 2016; Trabanco et al. 2019). Nevertheless, further investigation is needed to elucidate if different treatment regimens or adjunct treatment of mGlu2 PAMs would provide benefit for schizophrenia therapy (Litman et al. 2014).

17.5 Interactions Between Glutamatergic and Serotonergic Systems in Schizophrenia: The Functional Heterocomplex 5-HT2A-mGlu2 as a New Target

Both serotonergic and glutamatergic neurotransmitter systems have been involved in the pathophysiology of schizophrenia. The interaction between these systems is supported by extensive evidence that demonstrates its functional relevance in schizophrenia, particularly through the heterocomplex formed by 5-HT2A and mGlu2 receptors (Moreno et al. 2016; Shah and Gonzalez-Maeso 2019).

The individual implication of each receptor comprising this heteromer has also been evidenced in schizophrenia (Muguruza et al. 2016; Selvaraj et al. 2014), and both receptors have been considered as targets for antipsychotic drug development. It has been demonstrated the existence of a specific functional heteromeric complex formed by 5-HT2A and mGlu2 receptors through which serotonin and glutamate ligands modulate the pattern of G protein-coupling in living cells (Baki et al. 2016; Gonzalez-Maeso et al. 2008; Moreno et al. 2012). The mechanism of action of both antipsychotic (Fribourg et al. 2011) and propsychotic hallucinogenic drugs

(Lopez-Gimenez and Gonzalez-Maeso 2018) requires this serotonin-glutamate heterocomplex. Hallucinogenic 5-HT2AR agonists, such as lysergic acid diethylamide (LSD) or 2,5-dimethoxy-4-iodoamphetamine (DOI), induce the activation of both Gq/11 and Gi/o protein signaling pathways, while non-hallucinogenic 5-HT2AR agonists only activate the Gq/11 route (Lopez-Gimenez and Gonzalez-Maeso 2018). The existence of the functional expression of 5-HT2A/mGlu2 heterocomplex is necessary for the hallucinogenic-dependent Gi/o activation and behavioural effects (Moreno et al. 2011; Moreno et al. 2012). Moreover, it has been reported that the signaling inputs mediated by atypical antipsychotic drugs, such as clozapine and risperidone, which are high-affinity antagonists at the serotonin 5-HT2AR, are indeed integrated by the 5-HT2AR/mGlu2R heterocomplex that modulates signaling outputs and behavioural changes (Fribourg et al. 2011). Thus, the balance of Gi/o and Gq/11-dependent signaling of serotonergic and glutamatergic drugs would be mediated by the 5-HT2AR/mGlu2R heterocomplex and this activity would predict the propsychotic or antipsychotic effects of different pharmacological compounds (see Fig. 17.2).

Altered density of 5-HT2A and mGlu2 receptors has been found in postmortem PFC of schizophrenic subjects, with increased 5-HT2AR and decreased mGlu2R binding densities (Gonzalez-Maeso et al. 2008; Muguruza et al. 2013). Furthermore, the ligand binding crosstalk between 5-HT2A and mGlu2 receptors was found dysregulated in the postmortem PFC of schizophrenic subjects when compared with controls (Moreno et al. 2012). Additionally, the PFC signaling pattern of

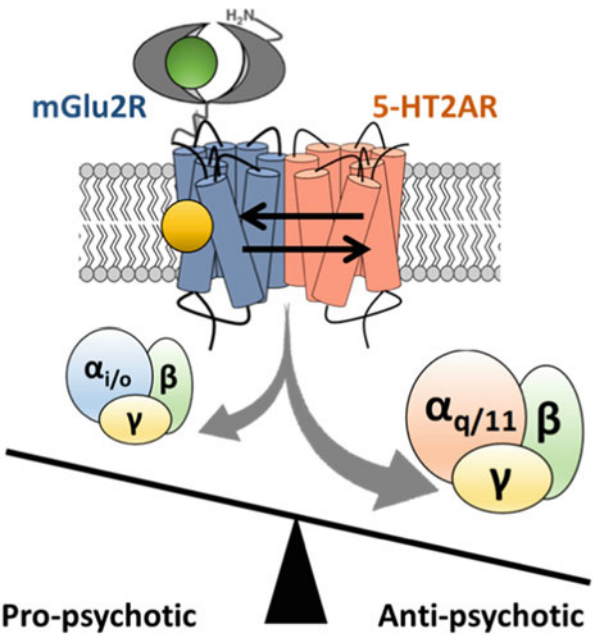

Fig. 17.2 Scheme of 5-HT2AR/mGlu2R heterocomplex activation of both Gq/11 and Gi/o proteins. The binding of orthosteric (green circle) or allosteric (yellow circle) agonists to the mGlu2 receptor could differentially modulate Gi/o and Gq/11 coupling through the heterocomplex. The thrust of this balance towards the non-hallucinogenic signaling pathway Gq/11 could predict potential antipsychotic effects of these drugs

mGlu2Rs has been shown altered in schizophrenia, with a significant reduction in the mGlu2R-dependent activation of Gq/11, but not Gi/o proteins (Moreno et al. 2016). Furthermore, a supersensitive 5-HT2AR signaling through inhibitory Gi1 proteins has been reported in the PFC of schizophrenia subjects, suggesting a dysfunctional pro-hallucinogenic agonist-sensitive 5-HT2AR conformation (Garcia-Bea et al. 2019).

It has been demonstrated that chronic atypical antipsychotics downregulate the transcription of mGlu2R through epigenetic modifications (Ibi et al. 2017; Kurita et al. 2012). In this sense, the ineffectiveness of mGlu2/3 receptor agonists in schizophrenic patients that were previously treated chronically with atypical antipsychotic drugs (Kinon et al. 2015)—that have a prominent 5-HT2AR antagonist activity—could be a result of the downregulation of mGlu2Rs derived from a functional crosstalk with 5-HT2ARs at the epigenetic level (Ibi et al. 2017).

Pharmacogenetic studies have also linked different SNPs in the gene encoding the 5-HT2AR and patients response to pomaglumetad in clinical trials. Thus, patients carrying the T/T genotype at rs7330461 were consistently associated with an increased treatment response to pomaglumetad (Liu et al. 2012; Nisenbaum et al. 2016). Overall, these facts point to a putative role of the 5-HT2AR/mGlu2R heterocomplex in the antipsychotic-like properties of the mGlu2R agonists and/or PAMs that could explain the controversial results reported in clinical trials (Muguruza et al. 2016).

17.6 Conclusion and Future Perspectives

Despite the reformulations and updates on the dopamine hypothesis of schizophrenia, its aetiopathology cannot be explained based solely upon dopaminergic dysfunction. Most of the current available antipsychotic drugs are based on a dopaminergic blockage that ameliorates positive psychotic symptoms with no impact on negative symptoms or cognitive deficits. Thus, many patients with schizophrenia remain persistently disabled. Glutamatergic hypothesis of schizophrenia accounts for the three clinical dimensions of the disease and it leads to new treatment approaches specifically targeting the unmet medical needs. Despite discouraging results from the two glutamatergic-based compounds that reached phase III development in clinical trials (bitopertin and pomaglumetad), the accumulating postmortem, genetic and neuroimaging evidence suggests that glutamatergic approaches to treat schizophrenia still need to be developed, but special attention must be placed on how clinical trials are designed and conducted (Beck et al. 2016). Undoubtedly, clarifying if specific subgroups of patients—depending on their specific pathophysiology, genetics or previous medication history—could benefit from using these new glutamatergic drugs deserves further research.

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Chapter 18
Glutamate and Epilepsy: An Insight from Temporal Lobe Epilepsy

Alberto E. Musto

Abstract The normal physiology of neurons is achieved through a delicate balance between excitatory and inhibitory synapses. Any disruption of such intricate balance can induce neuronal hyper-excitability in a susceptible neuronal network leading to seizures. Glutamate is the main excitatory neurotransmitter in the nervous system but when it is accumulated in excess induces neurotoxicity, mediates neuronal hyper-excitability and seizures. In epilepsy, glutamate is abnormally concentrated in the brain. Such abnormality is related with an altered synthesis, metabolism, storage, exocytosis, and clearance of glutamate. Accumulation of glutamate triggers over-activation of ionotropic *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (Kainate) receptors (KAr) as well as metabotropic glutamate receptors (mGLURs) in neurons. In addition, the abnormal molecular signaling from those receptors promotes cell damage and aberrant neuronal plasticity that could participate in the biology mechanism of epilepsy. In addition, glutamate is accumulated in other neurological with high incidence of recurrent epilepsy, such as glioblastoma multiforme and Sturge-Weber syndrome. Dissecting the cellular and molecular biology of glutamatergic system in inducing, propagating, and sustaining seizures will provide a new avenue for an innovative anti-seizure and/or anti-epileptogenenic drugs.

Keywords Glutamate · Epilepsy · Seizures · NMDA · AMPA · Kainate · Epileptogenesis

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_18

18.1 Introduction

The normal physiology of neurons is achieved through a delicate balance between excitatory and inhibitory synapses (Kandel 2013). Any disruption of such intricate balance can induce hyperexcitability in a susceptible neuronal network (such as those residing in neocortex, hippocampus, etc.) leading to seizure(s) (Musto et al. 2015; Musto et al. 2016). A seizure, therefore, represents a brief episode of involuntary altered neurophysiological function associated with an electrical abnormality within the brain, usually detected by an electroencephalography (EEG). Epilepsy, as defined clinically by The International League Against Epilepsy (ILAE), is a neurologic disease characterized by: "*(a) the recurrence of unprovoked and spontaneous seizures at different time points, at least more than 24 h apart; (b) one unprovoked or reflex seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years 3, or (c) a diagnosis of an epilepsy syndrome*" (Fischer et al. 2014).

Epilepsy has no cure except for a small population of patients that require surgical resection of the epileptic zone. Most patients respond satisfactorily to a clinical treatment; however, almost 20–30% of them become resistant to current drugs. This is often the case with temporal lobe epilepsy (TLE) or limbic epilepsy, one of the most common forms of epilepsy in adults (Scharfman 2007; Bertram 2009). In addition to the adverse effect of anti-epileptic drugs, patients with TLE have an increased risk for early mortality and comorbidities including cognitive dysfunction, depression, and anxiety disorders. In addition, TLE brings social stigma and increases the costs of healthcare (Schmidt and Stavem 2009; Harroud et al. 2012).

Hayashi found that glutamate-anion of glutamic acid, if applied directly into the cerebral cortex induces clonic seizures (Hayashi 1952). Since then, scientists have been narrowing on glutamatergic system trying not only to understand its role in the normal neurophysiology (Zhou and Danbolt 2014), but also how glutamate participates in epilepsy (DiNuzzo et al. 2014; Kanamori 2017).

Glutamate is the main excitatory neurotransmitter in the nervous system, when it comes to participating in neuronal organization during development and experience-dependent plasticity (Kandel 2013). Physiologically, extracellular concentrations of glutamate in the neural tissue are low compared to either intracellular or subcellular sites (Burger et al. 1989; Herman and Jahr 2007; Morales-Villagrán et al. 2016). But, when glutamate is accumulated in excess in the extracellular space of neural tissue, it induces neurotoxicity (Zhou and Danbolt 2014) as well as mediates hyper-excitable neuronal responses to a physiological input and promotes aberrant neural plasticity (Ben-Ari 1985; Scharfman 2007) depending on the level of its accumulation and permanence in the neural tissue.

In epilepsy, glutamate is abnormally concentrated in the extracellular compartment of the neural tissue (During and Spencer 1993). High levels of glutamate have been found in the epileptic tissue before and during seizures (Çavuş et al. 2016; Herman and Jahr 2007), in the cerebral spinal fluid from patients with newly

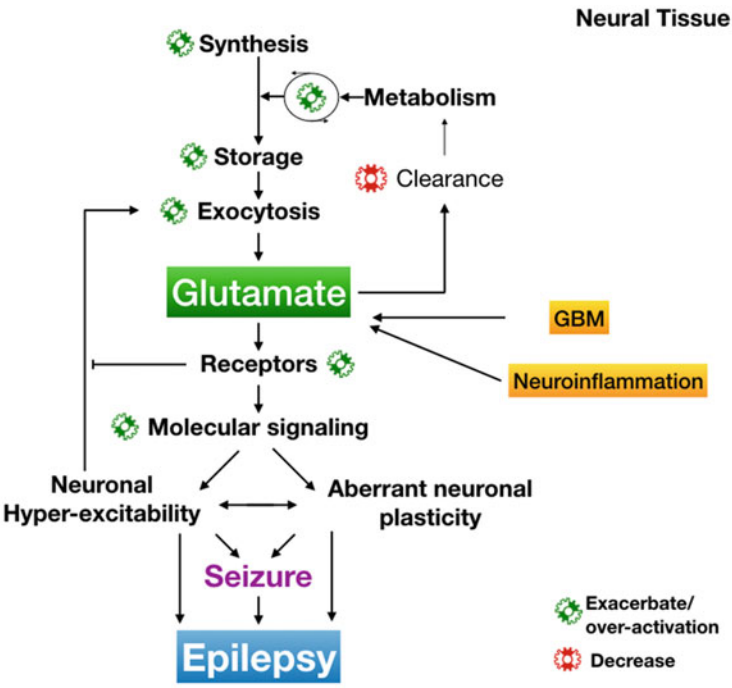

Fig. 18.1 Glutamate system in epilepsy: Exacerbation or deficit of the neurobiological mechanism of each step (synthesis, storage, etc.) of the glutamate system can contribute to increases of glutamate in the neural tissue and as a consequence promotes neuronal hyper-excitability and aberrant plasticity which in turn mediates seizures and epilepsy. In addition, glioblastoma multiforme (GBM) or neuroinflammation mediated by stroke, traumatic brain injury, or Alzheimer's disease contributes to an increase in glutamate and then increases seizure susceptibility and epilepsy

diagnosed epilepsy (Kälviäinen et al. 2006) and epileptic foci (Davis et al. 2015). Using an experimental model of epilepsy, the extracellular accumulation of glutamate during seizures had been shown to correlate with an increase in the fire rate of neurons, the amplitude of local field potentials (Li et al. 2018), and electroencephalographic oscillations during spontaneous epileptiform events (Morales-Villagrán and Tapia 1996).

Since the glutamate accumulation exerts cellular physiology through its receptors, those receptors have been considered as the main pharmacological targets to treat epilepsy (Rogawski and Löscher 2004). Hence, modulating the biology of glutamate is one of the focuses in drug development against epilepsy, especially in TLE.

In this chapter, the glutamate system in epilepsy (Fig. 18.1) is updated and summarized, providing perspectives for new avenues for new treatment in epilepsy and its application for mental health disorders (O'Donovan et al. 2017).

18.2 The Glutamatergic System in Epilepsy as a Disrupted Homeostatic Synaptic Scaling

The disruption of glutamatergic system involved in the development of seizure and epilepsy is related with: (A) an altered synthesis, metabolism, storage, exocytosis, and clearance of glutamate in neural tissue, and (B) an over-activation of ionotropic *N*-methyl-D-aspartate(NMDA),α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (Kainate) receptors (KAr) as well as metabotropic glutamate receptors (mGLURs) in neurons.

18.2.1 Altered Synthesis, Metabolism, Storage, Secretion, and Clearance of Glutamate

In epilepsy, the increase of glutamate in the brain could be related to one or more factors such as: increase of its synthesis which in turn increases its concentration in neurons, its accumulation in the presynaptic vesicles, an increase of its secretion from presynaptic terminals, or an impairment of its clearance from the extra synaptic sites.

The upregulation of the synthesis of glutamate in epilepsy is mediated by an increase of glutamine, which is metabolized to glutamate by phosphate-activated glutaminase or by the activation of tricarboxylic acid cycle (TCA) substrates. Both the enzyme and TCA substrates are abnormally increased in TLE (Eid et al. 2007; Melø et al. 2006). In addition, the activity of glutamate dehydrogenase, the enzyme which converts glutamate to α-ketoglutarate and has a critical function in neural metabolism (Kim and Baik 2019), is decreased in hippocampal tissue from a patient with TLE (Malthankar-Phatak et al. 2006). On the other hand, reduced ATP synthesis and oxidative stress increase the concentration of glutamate, thereby increasing the excitability of neurons (Dutuit et al. 2002). This particular cellular event could lead to sustained accumulation of glutamate during the inter-ictal period which is associated with a reduced metabolism in the neuronal tissue (Joo et al. 2004).

The other factor to increase the production of glutamate is through several transcription factors such as the Sonic Hedgehog (Shh). Shh is a mitogen and a morphogen, which plays a role in cell proliferation and differentiation during early development, rapidly enhancing extracellular glutamate levels (Feng et al. 2016). Shh expression increases during the evolution of epilepsy in an experimental model of TLE (Fang et al. 2011) suggesting an important role in limbic epileptogenesis.

Once the glutamate increases in the neuroplasma, glutamate is packaged into presynaptic vesicles to be released in the synaptic cleft, as a consequence of either action potential transmission or activation of mGlu2–3 receptors which result in calcium influx (Sceniak et al. 2012, see below NMDA receptors). In epilepsy, the entrance of glutamate to presynaptic vesicles through vesicular glutamate transporters (VGLUT1–3) is increased in the hippocampus (Contreras-García et al.

2018). Also, in some cases of refractory epilepsy SNAREs proteins, which mediate vesicle docking; priming; fusion; and neurotransmitter release into the synaptic cleft, are either mutated (Rohena et al. 2013) or increased in expression (Xiao et al. 2009) that could lead to an increase of loading of glutamate in the vesicles.

Synaptic vesicle glycoprotein 2 (SV2) is a prototype protein specifically identified in the synaptic vesicles of neurons which regulates exocytotic release of neurotransmitters (Chang and Südhof 2009; Ciruelas et al. 2019). Recently, (4R)-4-(2-chloro-2,2-difluoroethyl)-1-{[2-(methoxymethyl)-6(trifluoromethyl)imidazo [2,1-b][1,3,4]thiadiazol-5-yl]methyl}-pyrrolidin-2-one (Padsevonil) which binds to SV2 proteins as well as interacts with GABA A receptor at the benzodiazepine site had been shown to reduce seizure activity (Leclercq et al. 2020).

Moreover, altered molecular signaling related to glutamate exocytosis could be another factor that contributes to glutamate accumulation in extrasynaptic sites. This is the case of presynaptic c-Jun N-terminal kinase 2 and its interaction with Syntaxin-1a (JNK2/STX) which mediate glutamate release (Marcelli et al. 2019). In addition, activation of JNK molecular signaling is involved not only in neural hyper-excitability but also in the development of epilepsy (Cole-Edwards et al. 2006).

Homeostasis of glutamate is balanced between glutamate reuptake from the synaptic cleft by a series of glutamate transports, and proteins that initiate its recycling back to glutamine (O'Donovan et al. 2017), mainly through the physiology of astrocytes.

Extracellular glutamate is returned to the cellular compartment via transporter proteins in astrocytes and neurons using Na + −dependent excitatory amino acid transporters (EAATs), EAAT1 and EAAT2 are in human and glutamate aspartate transporter (GLAST) and glutamate transporter-1 (GLT-1) present in rodents) (Vandenberg and Ryan 2013). An impairment of glutamate uptake could sustain glutamate-activated receptors that lead to neuronal hyper-excitability and seizures (Eid et al. 2016). The downregulation or dysfunction of astrocyte GLT-1 also plays a role in epileptogenesis (Peterson and Binder 2019; Muñoz et al. 2019).

On the other hand, inhibition or loss of astrocytic glutamine synthesis impairs glutamate synthesis disrupting the glutamine metabolism which in turn promotes glutamate accumulation and its potential to activate a mechanism underlying recurrent seizure (Mayer et al. 2020).

However, the extracellular concentration of glutamate could have some fluctuation depending on when and under which circumstances the glutamate is measured. For example, during the second seizure episode diminished glutamate biosynthesis, enhanced glutamate reuptake, and/or neuronal death are considered possible causes of the attenuated glutamate release (Furness et al. 2019).

These observations suggest that abnormal increments of glutamate in the neural tissue sustain with time, contributing to over-excitability of neuronal networks and as a result epileptiform discharges occur leading to seizures (Albrecht and Zielińska 2017).

Although it is not clear if one or more factors of the altered biology of glutamate mentioned before are enough for the development of epilepsy, there is clear evidence

from the clinical and experimental studies that they provide conditions which are favorable for spontaneous recurrent seizure states (Wang et al. 2015).

18.2.2 Function of Different Glutamate Receptors and Its Molecular Signaling Are Altered in Epilepsy

One of the main consequences of the abnormal accumulation of glutamate in the brain is the over-stimulation of NMDA receptors (Wang and Qin 2010). These inotropic calcium-permeable receptors, voltage-dependence, are modulated by glycine, Mg2+, and seven subunit proteins (Wang and Furukawa 2019), their amino acid sequence of the protein domains, and the scaffolding proteins in the synaptic membranes. These receptors can be removed from the synaptic membranes by enzymatic action and endocytosis mechanism (Hansen et al. 2017). NMDA receptors can contribute to hyper-excitability of pyramidal neurons (Banerjee et al. 2015). In epilepsy, NMDA receptors are rearranged through the upregulation and downregulation of the subunits following epileptic seizures (Mihály 2019). One of its subunits, NR2B, is upregulated in the hippocampal formation and entorhinal cortex in experimental epilepsy (Zubareva et al. 2018), enhancing seizure susceptibility by upregulating different molecular signaling such as cyclin-dependent kinase-like 5 (Okuda et al. 2017). In experimental epilepsy, subunit mutations of NMDA receptors are associated developments of seizures (Xu and Luo 2018). For example, genetic modifications of subunit NMDA are related with epileptic syndrome (Von Stülpnagel et al. 2017). Therefore, the molecular biology of these receptors offers a great potential of molecular targets for epilepsy.

Experiments conducted to elucidate different types of receptor subunits and their molecular signaling involved in regulating the response to excessive glutamate in extracellular components have yielded the following results. For example, transmembrane protein 25 gene (Tmem25), upregulated in epilepsy mediates the expression of NR2B-a crucial subunit in NMDA receptor, which is phosphorylated at Tyr1472, increases receptor excitability (Yang et al. 2019; Zhang et al. 2019). Another example is the miR-139-5p downregulation, that enhances the expression levels of NR2A (Alsharafi et al. 2016) when interacting with NR2B, can mediate development of seizures (Tang et al. 2017). In addition, peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1), which inhibits NR2A-NR2B interaction, is decreased remarkably in epilepsy (Tang et al. 2017).

In addition, zinc had been proven to be an allosteric inhibitor of those subunit receptors and, therefore, a crucial modulator of excitatory synaptic transmission (Dudek 2001; Serraz et al. 2016). However, how increased glutamate levels in synapse alter intracellular zinc levels is yet to be elucidated.

Additionally, NMDA interaction with other receptors can potentiate glutamate-induced seizures. This is the case with sCaV3.2 channels, a T-type calcium channel encoded by CACNA1H which regulates NMDA receptors (Wang et al. 2015).

ASIC3 receptor is another example which, if inhibited, exacerbates seizures through its interaction with the NMDA receptors via CaMKII/CREB signaling pathway (Çavuş et al. 2016).

Glutamate accumulation triggers over-activation of AMPA receptors which, in turn, mediate neuronal hyper-excitability and seizures (Rogawski 2013; Egbenya et al. 2018). AMPARs are ligand-gated ion channels with four different subunits, GluR1–4 wherein activation dependent upon phosphorylation of AMPAR serine site and modulation of GRIP1 and PICK1, the classical auxiliary subunits TARP and CNIH, can mediate seizures (Bissen et al. 2019).

Epileptiform activity leads to an increase in the amplitude of AMPA receptors-mediated postsynaptic potentials (Amakhin et al. 2018). Over-activation of AMPA could be the consequence of an increased expression of the GluA1 subunit at the postsynaptic membrane (Rajasekaran et al. 2012). Any mutation in its submits, for example in GluA, can mediate epileptic seizure (Itoh et al. 2018). In addition, loss of function mutations in a human AMPA receptor are associated with choreoathetosis, cognitive deficits, and epileptic encephalopathies (Stewart et al. 2019). Specifically, downregulation of subunits GluA2 (Lorgen et al. 2017) and/or GluA1 (Lopes et al. 2015) induces epilepsy. On the other hand, phosphorylation of GluR1 S831 and S845 affects seizure susceptibility and excitability (Rakhade and Loeb 2008).

Intraperitoneal administration of AMPA receptor antagonists within 48 h of early-life seizures reduced later-life seizure susceptibility and hippocampal neural injuries (Rakhade and Loeb 2008). In addition, AMPA receptor inhibitors (such as Perampanel), competitive antagonist, ion-channel blockers, and negative allosteric modulators have been developed against epilepsy (Rogawski 2013). For example, decanoic acid which binds sites on the M3 helix of the AMPA-GluA2 transmembrane domain, independent from the binding site of perampanel, acts as a non-competitive antagonist and mediates anti-seizure effects (Chang et al. 2016).

ATAD1, ATPase Family AAA Domain Containing 1, mediates AMPA receptor recycling. In ATAD1 knockout mice reversed behavioral defects, normalized brain MRI abnormalities, prevented seizures, and prolonged survival. The ATAD1 patients treated with Perampanel showed improvement in hypertonicity and resolution of seizures (Ahrens-Nicklas et al. 2017).

On the other hand, combinations of NMDA and AMPA antagonist receptors have been shown to attenuate epileptogenesis (Schidlitzki et al. 2017), but no clinical drug designed to antagonize both simultaneously is yet clinically available.

Glutamatergic influx in synapse is mediated also by KAr. KAr located in pre- and post-synapsis participates in postsynaptic depolarization of glutamatergic and GABAnergic neurons (Cossart et al. 2001).

KAr activation, either acute or chronic, induces seizures in models of epilepsy and depending on its activation can induce a spontaneous recurrent seizure state associated with neural tissue modification such as neural loss, astrogliosis, and hippocampal sclerosis of CA1 and CA3 pyramidal cells which resemble characteristics of human TLE (Ben-Ari 1985).

Among KAr subunits, the GluR5–7 subunit plays as homomeric and heteromeric receptors, while KA1 and KA2 subunits have an auxiliary role that when they can

associate with any of the GluR5–7 mediate neuronal excitability. In human and experimental epilepsy, there is increased expression of GluR5, 6,7, and KA1 and KA2 (Chittajallu et al. 1999) indicating an active role in neuronal hyper-excitability.

On the other hand, there is impairment of the inhibitory network resulting from the depression of GABAergic interneuron transmission facilitated by activation of KAr subunit, GluK1 (Girard et al. 2019). In contrast, GluK2 subunit, located in excitatory neurons, modulates glutamate release presynaptically (Rodríguez-Moreno and Sihra 2013) and then induces neuronal hyper-excitability (Castillo et al. 1997; Schmitz et al. 2001) leading to seizures.

In addition, kainate subunits participate in the formation of aberrant neural synapses observed in the hippocampus as a consequence of recurrent seizures (Falcón-Moya et al. 2018). Since most of those aberrant terminals are excitatory, this cellular modification induced by KAr could exacerbate the intra- or extracellular accumulation of glutamate in epilepsy.

The Neuropilin and tolloid-like protein 1 (NETO1), a protein involved in the development and/or maintenance of neuronal circuitry, is required for formation of KAr containing synapses in interneurons and it has been postulated as a potential therapeutic target for treatment of seizures (Orav et al. 2019).

Selective KAr antagonism, specially the GLUR6 signaling, shows promise in epilepsy; however, drugs are still in development (Kaminski and Henley 2007).

mGLUR could play a role in epilepsy (Ali et al. 2016). mGluRs are G-protein coupled receptors divided into three groups, including eight subtypes (mGluR1–8). (Hermans and Challiss 2001). Neuronal excitability is accentuated via mGLUR activation which increases glutamate exocytosis by activating phospholipase C and protein kinase C (Group I).

mGluR5 signaling during TLE development mediates glutamate uptake in the hippocampus (Ure et al. 2006) and together with the mGlu7 subunit, which reduces spontaneous seizures (Girard et al. 2019), is postulated as a target for epilepsy treatment (specifically, for absence epilepsy and propagation of seizures). In addition, blocking mGluR5 protects the brain after seizures (Jesse et al. 2008); however, its role in TLE is discussed (Kandratavicius et al. 2013).

On the other hand, mGLUR can reduce the release of glutamate through other subgroups by regulating adenylyl cyclase activity (Ure et al. 2006) (Group II). For example, any downregulation of mGluR2, 3 in limbic regions contributes to neural damage in the hippocampus during epileptogenesis (Bocchio et al. 2019) suggesting that these mGLURs inhibit the release of glutamate into human neocortical pyramidal neurons, regulating network excitability.

Overall, the over-activation of glutamate receptors and the consequences of its molecular signaling can contribute to neural hyper-excitability or to increased inhibition in the neural excitability showing a complex dynamic response to glutamate accumulation. The time and cellular location of this receptor activation and its role in the initiation, maintenance, or adaptation of seizures need to be elucidated to determine the effective therapeutic target without adverse effect in epilepsy.

18.3 Glutamate in Neurological Disorders Related with Epilepsy

Glutamate is elevated in the neural tissues obtained from neurological pathologies with high incidence of recurrent epilepsy, such as glioblastoma multiforme (GBM) (Neal et al. 2018) and Sturge-Weber syndrome (Juhász et al. 2016). In the case of GBM, malignant cells release glutamate in the extracellular space via Xc- antiporter system, which then accumulates abnormally high, resulting in seizure induction (Mayer et al. 2020). Cystine/glutamate antiporter system (xCT) is proposed as a new target for antiepileptogenic treatments due to its crucial roles in glutamate homeostasis and neuroinflammation. xCT is a pro-convulsive factor in glioma-associated seizures setting. Drugs targeting it, such as sulfasalazine, have shown to decrease seizure susceptibility and limit recurrent seizures in experimental models of epilepsy (De Bundel et al. 2011).

Neuroinflammation is another factor that contributes to the development of epilepsy (Rana and Musto 2018). Neuroinflammation indirectly perturbs neuronal activity by promoting glutamate accumulation in extracellular space and hence contributing to seizure susceptibility and synchronizing the neuronal network. Neuroinflammation is a common denominator in neurological conditions such as stroke, traumatic brain injury, and Alzheimer disease that are related with epilepsy. For example, activation of microglial can mediate production of pro-inflammatory molecules and hence, enhance glutamatergic action in the synapse. Increase of pro-inflammatory mediators in neural tissue, such as IL-1 beta, platelet activating factor (Musto et al. 2016), and TNF alpha induces glutamate release by activating microglia and hence, thought to be involved in the etiology of neuronal hyperexcitability (Rana and Musto 2018).

18.4 Glutamate as a Biomarker for Seizures and Epilepsy

Glutamate levels are proposed as a biomarker for the development of post-stroke epilepsy (Neal et al. 2016). [11C]ABP688 is a radio-ligand which binds specifically to the mGluR5 allosteric site providing a focal biomarker for local epilepsy, such as MTLE (Çavuş et al. 2016). In addition, [18F] GE179 is a promising PET probe to image functional NMDA receptor alterations, which can be applied in epilepsy.

18.5 Conclusion and Future Perspectives

Without a doubt, the glutamatergic system is impaired in epilepsy. Its complex physiological upregulation and downregulation is drastically altered during epileptic disease, requiring multidisciplinary approaches to treatment.

Dissecting the cellular and molecular biology of the glutamatergic system in inducing, propagating, and sustaining seizures will provide a new avenue for an innovative anti-seizure and/or antiepileptogenic drug.

Acknowledgments Deepak Sharma and Maumita Das for their revision and comments of the manuscript.

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Chapter 19
Glutamate Function in Anxiety Disorders and OCD: Evidence from Clinical and Translational Studies

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Abstract Anxiety disorders affect a significant number of the world population. They are among the most prevalent disorders in society and impose enormous suffering and disabilities on the people affected. Among the anxiety disorders described in DSM-5, the most prevalent and studied are generalized anxiety disorder (GAD), social anxiety disorder (SAD), and panic disorder (PD). Obsessive-compulsive disorder (OCD), although it shares many morphophysiopathological aspects and symptoms with anxiety disorders, is addressed in a separate topic in DSM-5. For all these disorders, serotonergic and dopaminergic neurotransmission seems to play an important role. However, considering that the circuit involved in these disorders has glutamate as a critical neurotransmitter, this chapter emphasizes glutamate in anxiety disorders and OCD. The relationship between glutamate and the hypothalamic-pituitary-adrenal axis (HPA) is addressed. Studies on the role of glutamate in disorders are considered, as well as the brain structures involved. The cortico-striated-thalamus-cortical (CSTC) and limbic areas connected to the circuit and involved in the OCD are addressed, highlighting the function of glutamate in connections, and possible interactions with other neurotransmitters. In OCD, genetic studies about genes underlying glutamatergic neurotransmission are also considered. Also, in both anxiety disorders and OCD, adjunctive treatment strategies with glutamatergic compounds are addressed.

Keywords Glutamate · Anxiety disorders · Obsessive-compulsive disorder · Cortico-striatal-thalamo-cortical (CSTC) circuit · Serotonin · Dopamine · GABA

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_19

19.1 Introduction

According to the DSM-V, anxiety disorders are classified into generalized anxiety disorder (GAD), social anxiety disorder (SAD), panic disorder (PD), separation anxiety disorder, selective mutism, specific phobia, agoraphobia, substance/medication-induced anxiety disorder, and anxiety disorder due to another medical condition (American Psychiatric Association 2013).

Excessive and persistent anxiety is predominant concerning daily activities in GAD. These feelings occur on most days for at least 6 months. The anxiety generated has a distressing character of longer duration that causes significant suffering since there is great difficulty controlling worrying thoughts, which distracts attention away from daily activities. Women are twice as likely to develop GAD as men (American Psychiatric Association 2013).

SAD is characterized by fear, avoidance, and intense anxiety in social situations that involve the possibility of evaluation. This evaluation is not restricted to academic and professional moments, including situations in which the person faces new social interactions and is overly concerned about being judged negatively. Faced with these perceptions, the individual feels rejected, humiliated, or even has the feeling of offending other people. The fear and anxiety generated are disproportionate to the situation to which the individual is exposed, and these feelings must persist for at least 6 months (American Psychiatric Association 2013).

In PD, panic attacks are the hallmark of the disorder. These are unexpected and recurring conditions, making the individual apprehensive and concerned about the appearance of new attacks. The attacks are sudden and full of intense fear that reaches a maximum in a few minutes along with physical symptoms, such as palpitations, tachycardia, sweating, tremors or shaking, feeling of shortness of breath or suffocation, choking, chest pain or discomfort, nausea or discomfort abdominal, feeling of dizziness, instability, dizziness or fainting, chills or hot flashes, and paresthesia. PD is also accompanied by cognitive symptoms, such as derealization, depersonalization, fear of going crazy, and dying. Panic attacks are followed by at least a month of apprehension or concern about new attacks and the possibility of a loss of control, as well as the individual may present a behavioral change to avoid any activity that can trigger the attacks. It is worth remembering that attacks often occur for no apparent reason and can serve as a specifier for other disorders such as substance use, depression, and psychotic disorders (American Psychiatric Association 2013).

In addition to the anxiety disorders specified in the DSM-V, the obsessive-compulsive disorders (OCDs), although they have very particular characteristics, some of their characteristics maintain intimate relationships with anxiety disorders. Consequently, OCD was addressed in DSM-5 as a separate chapter and following anxiety disorders (American Psychiatric Association 2013).

Fear and anxiety are adaptive behaviors crucial to survival. However, on many occasions, the fear responses can become maladaptive, leading to a generalization of the process. In these situations, various stimuli present in the environment can elicit fear and anxiety behaviors, as observed in OCD. Studies in different lines of

evidence, such as physiological and behavioral, suggest that glutamate neurotransmission plays a relevant role in the pathogenesis of anxiety-related disorders (Riaza Bermudo-Soriano et al. 2012).

Anxiety disorders and OCD have in common the involvement of glutamatergic neurotransmission beyond other pathophysiological mechanisms and brain structures that may be more specifically related to some of these disorders. Some neurotransmitters involved with anxiety disorders are gamma-aminobutyric acid (GABA), serotonin, norepinephrine, neuropeptides, and glutamate. Glutamate has received attention more recently for its involvement in the neurobiology and treatment of anxiety disorders (Nasir et al. 2020).

Just as GABA is the main inhibitory neurotransmitter, glutamate is the primary excitatory neurotransmitter in the central nervous system. Both neurotransmitters are abundant in limbic system structures involving fear and anxiety (Bergink et al. 2004). After identifying glutamate as a neurotransmitter in 1959, many studies have highlighted the importance of the glutamatergic system in the pathophysiology and treatment of psychiatric disorders (for a review, see: Machado-Vieira et al. 2009).

Under normal conditions, glutamate plays a prominent role in synaptic plasticity, learning, and memory (Marcondes et al. 2020) and is involved in neural development, cell proliferation, and migration (McDonald and Johnston 1990). However, under pathological conditions, glutamate's exacerbated activity culminates in excitotoxicity, leading to cell death (Pittenger et al. 2007).

The action of glutamate occurs through specific receptors located on the cell membrane's surface, classified according to their pharmacological and functional properties (Sanacora et al. 2008). The function and regulation of glutamate levels require presynaptic and postsynaptic neurons and glial cells, characterizing the so-called tripartite synapse (Machado-Vieira et al. 2009). Its function occurs through ionotropic (iGluR) and metabotropic (mGluR) receptors (Riaza Bermudo-Soriano et al. 2012).

mGlu receptors are not exclusively in the synapse region, and when bind to glutamate, they activate a signaling molecule, the G protein, which is responsible for initiating an intracellular signaling cascade. There are eight different types of metabotropic receptors, which are into three subgroups: Group I (mGlu1 and mGlu 5), Group II (mGlu2 and mGlu3), and Group III (mGlu4, mGlu6, mGlu7, and mGlu8) (Pilc et al. 2008; Bhattacharyya and Chakraborty 2007). mGlu receptors are essential in modulating synaptic excitability (Niswender and Conn 2010; Harvey and Shahid 2012).

iGlu receptors are ion channels with selective conductance for $Ca2^{+}$ and Na^{+}. Once activated, an influx of these cations occurs, supporting the neuron depolarization. iGluRs are classified into N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), and kainate (Traynelis et al. 2010; Harvey and Shahid 2012).

Many events contribute to glutamatergic changes, such as increased levels of glutamate or changes in glial cells due to decreased extracellular glutamate reuptake by excitatory amino acid transporters (EAATs) (Kugaya and Sanacora 2005).

19.2 Glutamate and Hypothalamic-Pituitary-Adrenal Axis

There is the knowledge that psychiatric illnesses, mainly major depressive disorder (MDD), post-traumatic stress disorder (PTSD), and anxiety disorders, can be triggered by stress, a body reaction responding to a situation resulting in neuronal balance dysregulation. It is important to note that some individuals are resilient to stress to adapt to stressful events and are more resistant to developing psychopathology. Among the risk factors for developing psychiatric disorders triggered by external stressors are genetic predispositions, family inheritance, socio-environmental factors, early life stress, and chronic diseases. The glutamatergic pathway is involved in the mechanism of resilience to stress. However, studies are still needed to understand how this modulation occurs (Faye et al. 2018).

Stressful events trigger a normal physiological and behavioral response that activates the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis's activation culminates in the secretion of corticotropin-releasing hormone (CRH) from the hypothalamus with the subsequent secretion of adrenocorticotropic hormone (ACTH) by the pituitary gland. In this way, ACTH is released into the bloodstream to reach the adrenal cortex, resulting in glucocorticoids secretion, primarily cortisol. The release of cortisol usually occurs 30 min after exposure to stress, and the recovery of this stimulus takes place in 60–80 min. However, prolonged and constant exposure to stressful events leads to chronic stress that leads to persistent functional changes (Faye et al. 2018).

The glutamatergic neurotransmission in the PFC represents a mechanism by which stress influences neuronal processes related to affection and cognition. The glutamatergic function involving the PFC in the stress response seems to be related to direct acute and chronic action mediated by the HPA axis. Although there is strong evidence that dopamine disorders are involved in the adverse effects of stress, the activation of monoaminergic neurotransmission due to an abnormal stressor seems secondary to changes in cortical neurotransmission glutamate (Moghaddam 2002).

The pharmacological block of glutamate release or specific glutamate receptors has been shown to prevent atrophy induced by glucocorticoids such as cortisol. Thus, there is a possibility that glutamatergic antagonism plays a role in blocking hyperactive HPA axis systems in neuropsychiatric disorders, thus presenting a neuroprotective role (Mathew et al. 2001). Activation of rats' amygdala through microinjection of glutamate increased plasma corticosterone, and this increase seems to depend on the dose of CRH in the median eminence. Blocking CRH release through an NMDA antagonist can be anxiolytic properties by inhibiting CRH release in the amygdala. In regions of the hypothalamus and brainstem, however, the response was inhibited by systemic dexamethasone, suggesting that glutamate mediation on the HPA axis is sensitive to steroids (Mathew et al. 2001).

Among the mechanisms underlying the stress-induced changes in the HPA axis are neuroplasticity changes, especially in the hypothalamus. Stimulation of the bed nucleus of the stria terminalis (BNST) produced long-term suppression of the

evoked field potentials in the paraventricular nucleus (PVN) of the hypothalamus. The administration of a non-competitive NMDA receptor antagonist, MK-801, reversed the suppression of potentials in the PVN (Tartar et al. 2006). The authors infer that the high-frequency stimulation applied to BNST produces a fast and long-lasting inhibition of the NMDA receptor in PVN, possibly through an increase in BNST inhibitory control. This study suggests that inhibition of the NMDA receptor may regulate the stress response that occurs with the activation of the HPA axis (Tartar et al. 2006).

There is evidence that the excitatory synaptic transmission of glutamate increases from stress due to social isolation. It causes a dysregulation in the receptors' expression and function in the central nervous system, especially NMDA, resulting in excitotoxicity and brain damage (Sestito et al. 2011). In this sense, the stress seems to result in quite complex changes that mainly involve neurochemical and neuroendocrine systems, in addition to physiological, anatomical, and behavioral changes (Mumtaz et al. 2018).

19.3 Generalized Anxiety Disorder: GAD

Generalized anxiety disorder (GAD) is quite common, affecting over 6% of the population during their lifetime. It is a disorder that causes disability and becomes chronic in many people due to adverse effects and consequent withdrawal from treatment. Besides, GAD is often accompanied by comorbidities, such as major depressive disorder, panic disorder, and substance abuse, making treatment even more challenging (Maron and Nutt 2017; Schanzer et al. 2019).

Abnormalities in neurotransmission and inhibitory-excitatory balance are characteristics observed in GAD patients (Schanzer et al. 2019). Studies suggest that gamma-aminobutyric acid (GABA) deficits and increased excitatory neurotransmission of glutamate are mechanisms involved in GAD's pathogenesis. Changes in the binding of GABA to GABA-A receptors found in the medial prefrontal cortex, amygdala, and hippocampus, are related to anxiety and fear responses (Roy-Byrne 2005).

As with other anxiety disorders, glutamate has receipt highlight as a neurotransmitter that plays an essential role in GAD. Studies in humans and animal models bring evidence about the action of glutamate in limbic brain structures involved in anxiogenic responses to stressful events (Mathew et al. 2005; Dunayevich et al. 2008; Riaza Bermudo-Soriano et al. 2012; Sugiyama et al. 2012).

Studies performed by functional magnetic resonance imaging (fMRI) sought to evaluate the brain responses in GAD in the face of negative emotional stimuli. These studies observed that fearful facial expression occurs in parallel to activate limbic structures, such as the amygdala, a relevant region controlling fear and emotional reactivity, and the insula, responsible for internal physiological states (Schanzer et al. 2019).

A GAD's striking symptom is the stringent, persistent worry and without the need for any external signal or stimulus. This symptom was observed through neuroimaging that pointed to an adaptive inadequacy of the medial prefrontal cortex (mPFC). Also, the induction of worrisome stimuli in GAD patients activates the anterior and dorsal cingulate, brain regions involved in stimulus-independent mental activity (Paulesu et al. 2010).

A worrying stimulus triggered a robust increase in the PFC's activity in elderly individuals with GAD. Imaging exams also found greater activation in the left amygdala. The GAD individuals showed greater activation in the left region than the controls. The authors argue that the left amygdala is activated when linguistic emotional alert stimuli are processed (Mohlman et al. 2017). The scientific literature provides evidence that glutamate is the primary neurotransmitter in the bidirectional connections between PFC and the amygdala (Sah et al. 2003). Although still lacking clarification, studies suggest that traumatic stress influences the connectivity between mPFC and amygdala and that compromised connectivity may be secondary to trauma-induced changes in the prefrontal glutamatergic pathways (Ousdal et al. 2019).

The connectivity between the PFC and the amygdala seems responsible for regulating outputs from the amygdala to the hypothalamus and activating physiological responses to anxiety (Fig. 19.1) (Mohlman et al. 2017). Studies with animal models of anxiety have observed that the NMDA receptor agonist in the PVN induced an increase in sympathetic stimulation, with a consequent increase in blood pressure and heart rate. At the same time, an NMDA antagonist blocked such effects (Li et al. 2006).

19.4 Panic Disorder: PD

PD is one of the most investigated anxiety disorders, affecting 2.7% of the population per year and has a lifetime prevalence of up to 5% in the general population. It affects women twice as often as men and frequently develops in late adolescence or early adulthood, with the average age of onset being 28 years (Zulfarina et al. 2019).

Still, there is no known etiology, as it is characterized by a heterogeneous psychiatric disorder that is difficult to diagnose. Also, it is common for patients with PD to have other medical conditions such as respiratory and heart diseases, mental disorders due to substance abuse (alcohol, caffeine, cannabis, and cocaine), affective disorder, post-traumatic stress disorder (PTSD), obsessive-compulsive disorder, and social phobia. These comorbidities are usually associated with greater severity and a worse clinical course of the disorder (Kelly et al. 2015; Chen and Tsai 2016). It is worth remembering that some patients manifest only panic attacks (PA) resulting from another psychiatric disorder. Generally, it is possible to predict its occurrence in these cases, as they correlate to a specific triggering situation (Zulfarina et al. 2019).

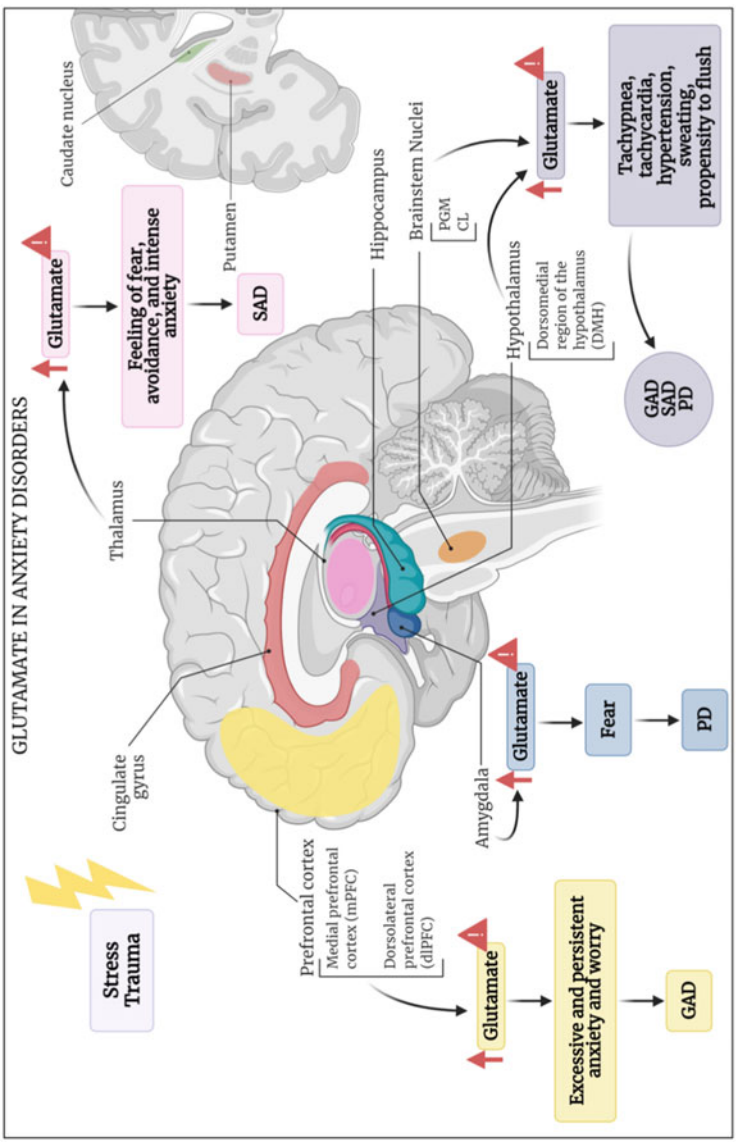

Fig. 19.1 Stress and trauma throughout life are involved in anxiety disorders. Among the brain structures highlighted are the prefrontal cortical areas, subcortical limbic areas, such as the hippocampus, hypothalamus, and amygdala, nuclei in the brain stem, such as the periaqueductal gray matter (PGM), cerulean locus (CL), in addition to the substantia nigra (SN) and ventral tegmental area (VTA). Glutamatergic hyperactivation in the amygdala, prefrontal cortex (PFC), and hypothalamus seems to be a critical mechanism in the imbalance of functional cortico-limbic activity and triggering generalized anxiety disorder (GAD), social anxiety disorder (SAD), and panic disorder (TP). Images were extracted from the BioRender app

Currently, the treatment of choice for PD includes selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and tricyclic antidepressants (TCAs), which can take several weeks to achieve the therapeutic effect. Therefore, therapy in combination with benzodiazepines (BZDs) is initially necessary. However, the drugs mentioned above do not show complete clinical results in the most treated patients (Perna et al. 2015).

An important mechanism that triggers PD is the inadequate activation of the central fear network, which includes the amygdala and its connections with the hippocampus, thalamus, hypothalamus, periaqueductal gray matter (PGM), cerulean locus (CL), and PFC. The PFC generates an inadequate interpretation of the sensory information and conditioning processes, resulting in panic attacks, anticipatory anxiety, and avoidance (Perna et al. 2015; Chen and Tsai 2016).

The metabotropic glutamatergic receptors were investigated to verify the regulation of presynaptic glutamate release and the consequent postsynaptic neuronal excitability in PD. It is possible to mention the Group II receptors (mGlu2 and mGlu3) that act negatively by modulating the release of glutamate and controlling GABA release and other neurotransmitters, including monoamines. A potent agonist of group II metabotropic receptors induces anti-panic-like responses in PD's animal model (Shekhar and Keim 2000). Another study noted that a selective allosteric potentiator of the mGlu2 receptor reversed panic-like behavioral and physiological changes in panic-vulnerable rats. The authors suggest that the best anti-panic effect with fewer adverse effects may occur because the selective activator mGlu2 acts by reducing the excessive action of glutamate without affecting the basal glutamatergic activity (Johnson et al. 2013). These receptors are found in the cortex, hippocampus, other limbic areas, and, to a lesser degree, in the brainstem and cerebellar regions and, thus, are involved in the anxiety, emotion, and cognition responses of animals and humans (Perna et al. 2011).

The dorsomedial hypothalamus (DMH) region coordinates neuroendocrine, autonomic, and behavioral responses to various homeostatic mechanisms (Johnson and Shekhar 2006). According to Perna et al. (2015), the decrease in inhibitory activity mediated by infusion of the inhibitor of GABA synthesis L-glycine (L-AG) in the HDM of rats results in the prolonged glutamate activity in this region. As a consequence, rats exhibit increased behaviors associated with panic, in addition to cardiorespiratory symptoms such as tachypnea, tachycardia, hypertension, and increased avoidance behavior in social interaction (Fig. 19.1) (Perna et al. 2015; Johnson and Shekhar 2012).

19.5 Social Anxiety Disorder: SAD

In social anxiety disorder (SAD), there is a global impairment of social functioning, with a prevalence of 1.9% to 12.1% in the general population. Among the neurobiological findings involved, the neurotransmitters serotonin, norepinephrine, glutamate, and GABA stand out, in addition to the neuropeptide oxytocin. Furthermore,

there is no single, comprehensive hypothesis available to explain SAD biological characteristics. It is common to have concomitantly other psychiatric disorders such as obsessive-compulsive disorder, generalized anxiety disorder, panic disorder, and body dysmorphic disorder (Marazziti et al. 2015).

Currently, the literature brings data that the neurobiology of SAD is related to the circuit of the basal ganglia and the reward and avoidance systems in general, amygdala, and hippocampus (Fig. 19.1) (Caouette and Guyer 2014). The reward/avoidance system is related to the basal ganglia, especially the caudate and putamen, the thalamus, and cortical regions, such as the anterior cingulate cortex (ACC) (Howells et al. 2015). Some important SAD behaviors arise from striatal hyperactivation (Lorberbaum et al. 2004; Guyer et al. 2014).

A study evaluated the concentrations of metabolites such as N-acetyl-aspartate (NAA), NAA with N-acetyl-aspartyl-glutamate (NAA + NAAG), glycerophosphocholine (GPC) with phosphocholine (PCh) (GPC + PCh), Myo-inositol, glutamate (Glu), and glutamate with its precursor glutamine (Gln) (Glu + Gln) in the ACC, in addition to also evaluating the metabolites NAA + NAAG and GPC + PCh in the cortical, striatal, and thalamic regions bilaterally. The protocol made a crossing of the metabolites with social anxiety measures and related symptoms of SAD patients. The researchers observed a decrease in the relative concentration of glutamate in the ACC of patients with SAD. Also, the NAA metabolite concentration seems to increase in the thalamus of SAD patients. Finally, the choline metabolite correlated with changes in social anxiety measures in the caudate and putamen (Howells et al. 2015). However, other studies seem to find opposites results. A protocol found a considerable increase in glutamate in the ACC, which was correlated with the intensity of SAD symptoms (Phan et al. 2005). Another study, in addition to a reduction in GABA and an increase in glutamate, found that levetiracetam, a compound that increases gabaergic activity, reduced SAD behaviors in parallel with a reduction in glutamine levels and an increase in GABA in the brain of SAD individuals (Pollack et al. 2008).

In this sense, it is understood that the role of glutamate in anxiety is due to the increase in glutamatergic transmission and the excessive release of glutamate in the limbic system. Therefore, patients with SAD showed an exaggerated activation of the limbic system in response to the social threat and situations that cause anxiety (Fig. 19.1) (Phan et al. 2005).

19.6 Anxiety Disorder and Glutamatergic Treatment

Glutamatergic neurotransmission as a therapeutic target for GAD has also been shown. Research with knockout mice for mGlu2 or mGlu3 has observed that a selective agonist's anxiolytic activity for these receptors requires the presence of one or both receptors (Linden et al. 2005). A multicenter clinical study found that treatment with the selective agonist mGlu2/3 promoted an anxiolytic effect with few adverse effects, corroborating preclinical research findings (Dunayevich et al.

2008). Activation of the mGlu2 and mGlu3 receptors is related to reduced presynaptic glutamate release or postsynaptic neuron hyperpolarization in limbic areas involved with anxiety, such as some amygdala nuclei, prefrontal cortex (PFC), and hippocampus (Schoepp et al. 2003).

Studies in animal models provide evidence that riluzole, a presynaptic inhibitor of glutamate release, has an anxiolytic effect (Stutzmann et al. 1989). The anxiolytic effects of riluzole are in different mechanisms and without the adverse effects of benzodiazepines (Sugiyama et al. 2012). Adult GAD patients treated for 8 weeks with riluzole experienced a rapid and sustainable reduction in anxiety symptoms. According to the Hamilton Anxiety Assessment Scale (HAM-A), approximately half of the treated individuals achieved remission. Noteworthily, the side effects were tolerable, allowing further studies to improve anti-glutamatergic agents' use in GAD treatment (Mathew et al. 2005). In another study using proton magnetic resonance spectroscopy (1H MRS), the researchers found a strong correlation between reduced anxiety symptoms and increased N-acetyl aspartate (NAA) concentrations in the hippocampus of patients treated chronically for 8 weeks with riluzole. Most patients who responded to riluzole had a considerable increase in hippocampal NAA at the end of treatment than baseline. On the other hand, patients who did not respond to treatment showed no difference or had a reduction in the concentration of NAA in the hippocampus (Mathew et al. 2008).

Proton magnetic resonance spectroscopy (1H MRS) is a non-invasive technique that allows assessing changes in specific metabolites in brain tissue resulting from pathological changes (Ramin et al. 2003; Hill and Olga 2014). NAA, a marker of neuronal viability, is one of the critical metabolites synthesized in brain tissue mitochondria and is present in neuronal bodies and axons. It is a marker that indicates neuronal density and viability (Ramin et al. 2003). The researchers suggest that riluzole on GAD is related to reducing glutamatergic excitotoxicity and increasing hippocampal plasticity (Mathew et al. 2008). In this regard, other studies have observed a reduction in hippocampal volume in GAD patients. Treatment with riluzole increased the hippocampal volume, positively correlated with NAA, and reduced anxiety symptoms (Abdallah et al. 2013).

Research on glutamatergic ionotropic receptors is still limited because of the significant adverse effects of binding substances. However, some research shows the anxiolytic effects of substances antagonizing NMDA and AMPA receptors (Wieronska et al. 2011). In a study with a fear-potentiated startle model in rats, the researchers observed that blocking the NMDA receptor in the caudal pontine reticular nucleus attenuated the fear-potentiated startle, suggesting that the potentiated response is dependent on the activation of NMDA receptors in this region (Fendt et al. 1996). Noteworthy were the studies, which showed the acute and long-lasting effect of ketamine, an NMDA receptor antagonist, in GAD patient refractory to other treatments. The individuals had few and tolerable adverse effects. The authors suggest ketamine as a potential and safe treatment strategy for individuals with resistant anxiety disorders (Glue et al. 2017).

Regarding glutamate as a therapeutic target in the SAD, it is noteworthy one study, which found that the infusion of ketamine promoted an anxiolytic response

for 2 weeks after administration in SAD individuals (Taylor et al. 2018). However, considering the therapeutic strategy, the function of glutamate seems to have paradoxical effects. Worthwhile notes the studies about this issue, which observe the effect of D-cycloserine on learning that seems to facilitate the extinction of fear. In this sense, it is worth mentioning that D-cycloserine, a partial NMDA agonist, has been showing beneficial effects, increasing the therapeutic response of exposure psychotherapy and, thus, helping to extinguish fear (Davis et al. 2006). Considering that D-cycloserine is a partial agonist, a question is possible: its action may occur as an agonist in some regions but as an antagonist in other regions of the circuit? D-cycloserine administered directly to the amygdala's basolateral nucleus facilitated the learning of fear extinction, and its action was dependent on the synthesis of proteins involved in signaling pathways that increase synaptic plasticity.

Another noteworthy piece of information is that D-cycloserine acts on the glycine site and modulates the NMDA receptor. Thus, the influx of calcium occurs in an eased way. Conversely, direct receptor agonists may be neurotoxic by increasing unregulated calcium input (Yang and Lu 2005). Still, another relevant explanation is that the glycine site is also activated by D-serine, an endogenous ligand more potent than D-cycloserine. Therefore, in regions of the circuit responsible for the fear acquisition, glycine and D-serine would already be saturated, surpassing D-cycloserine's action. This consideration is relevant since the acquisition of fear is an adaptive mechanism for safety and survival (Davis et al. 2006).

More recent studies corroborate the role of D-cycloserine in enhancing the effects of exposure therapy. Besides, D-cycloserine can also act in the consolidation of extinction. However, some studies have observed that D-cycloserine can act inversely in the consolidation process when individuals exposed to fear extinction therapy do not show therapeutic success in extinction memory (Hofmann et al. 2015).

19.7 Obsessive-Compulsive Disorder: OCD

According to DSM-V, Obsessive-Compulsive Disorder (OCD) is characterized by the presence of obsessions and compulsions (American Psychiatric Association 2013). Obsessions concern recurring and persistent thoughts, impulses, or images experienced in an unpleasant and intrusive way. On the other hand, compulsions are repetitive behaviors or mental acts that an individual feels obliged to perform due to an obsession or according to rules that must be strictly applied. OCD affects 1.1–1.8% of the population worldwide. Males are the most affected in childhood, and females are most affected in adulthood (American Psychiatric Association 2013).

The DSM-V approaches OCD jointly with other related disorders. The topic in DSM-V includes OCD, accumulation disorder, body dysmorphic disorder, skin-picking disorder, trichotillomania, OCD and substance/drug induction disorder, OCD and related disorder due to some medical condition, OCD and specified related

disorder and OCD and unspecified related disorder, for example, repetitive behavior disorder with a focus on the body and obsessive jealousy (American Psychiatric Association 2013).

There is extensive literature addressing the vast range of factors and mechanisms that are related to the expression of OCD. Among these factors and mechanisms are neurotransmitters glutamate, serotonin, dopamine, GABA, besides biological vulnerability as biological sex and genetics factors, and endogenous processes like neuroinflammation and oxidative stress (Woody et al. 2019).

Several hypotheses explain the pathophysiology of OCD. However, there is no consensus about the etiology, which is possibly heterogeneous. The scientific literature has pointed to glutamatergic neurotransmission as one of the critical mechanisms involved in OCD. Studies bring evidence that the levels of glutamate in the cerebrospinal fluid were elevated in OCD individuals (Chakrabarty et al. 2005).

The evidence supporting glutamate involvement in OCD includes genetic studies, pharmacological investigations, animal models, neurochemical studies, small clinical studies, and case reports (Pittenger et al. 2011).

In addition to the heterogeneity of factors, studies suggest a complex circuitry involving the frontal cortex, basal nuclei, thalamus, and other regions of the limbic system, when they do not process information in a balanced way, may trigger specific symptoms of OCD. The functional connectivity of the cortico-striatal-thalamo-cortical circuit is extensively investigated, and research suggests that deregulations in glutamatergic activity are critical in OCD (Karthik et al. 2020).

19.7.1 OCD and Glutamate in the Cortico-Striatal-Thalamo-Cortical (CSTC) Circuit

The relevance of the CSTC circuit in the pathophysiology of several types of disorders is not recent. Penney and Young (1983) bring critical explanations on the central role of the base's ganglia within the neuronal circuit CSTC, mainly in the modulation of behaviors. Studies with structural and functional neuroimaging data suggest that the CSTC circuitry plays an essential role in the pathophysiology of OCD. The imbalance in the CSTC circuits is well documented as a possible trigger of OCD. These circuits are composed of direct and indirect pathways, which act through the neurotransmitters glutamate and GABA. Both neurotransmitters are in balance in the absence of disorders (Saxena and Rauch 2000; Karthik et al. 2020). In OCD individuals, there is often an increased metabolic activity in both PFC and striatum, with a positive correlation between the two structures, suggesting glutamatergic cortical hyperactivity (Carlsson 2001).

The CSTC pathway originates in specific regions of the frontal cortex, including the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and also the dorsolateral prefrontal cortex (dlPFC). From these

cortical areas, the circuit reaches the corresponding striatal targets and then the thalamus. Finally, the circuit's signals return to the cortical areas, where the signals started (Milad and Rauch 2012; Zhu et al. 2015). The mPFC/ACC is involved in the affective and cognitive function directed to motivated behaviors, while the OFC seems responsible for integrating emotional and limbic information into behavioral responses (Chen et al. 2019). Meanwhile, considering the extinction of fear and behavioral inhibition, Milad and Rauch (2012) propose a circuit that considers specific cortical functions and involves limbic areas beyond the segregated cortico-striatal pathway. The study by Wood and Ahmari (2015) sought to investigate the role of other regions involved in the circuit of OCD. Among the regions covered are the medial OFC, the amygdala, and the ventral tegmental area (VTA) projections to the ventral striatum (VS). Among these, VS demonstrated a prominent role in processing affection and reward, being essential for integrating compulsive behaviors. Affective dysregulation and information processing from medial OFC, VTA, and the amygdala to the VS are mechanisms involved in OCD symptoms. Thus, other brain regions outside the classical CSTC pathway play a role, integrating the OCD circuit (Menzies et al. 2008). In addition to the greater complexity, the age, period of the symptoms, and the different dimensions of the symptoms with aggressive obsessions and compulsions may culminate in differences in the circuit structures' activities (Stein et al. 2019).

A meta-analysis provides evidence that during emotional processing in OCD individuals, there is an increase in the activation of the bilateral amygdala, right putamen, OFC, extending into the ACC and ventromedial PFC (vmPFC), middle temporal and left inferior occipital cortex. It is noteworthy in these studies that medication, the severity of symptoms, and comorbidities with other disorders promoted differentiated activation of brain structures (Thorsen et al. 2018). Another meta-analysis study showed that OCD patients showed overactivation of structures in neuronal circuits involving salience and emotional alert responses, such as ACC, insula, caudate head, and putamen. Conversely, in cognitive control regions, such as mPFC and posterior caudate, OCD individuals are under-activated (Rasgon et al. 2017). These studies corroborate the morphofunctional complexity of the circuitry.

Several neurotransmitter systems are found within CSTC circuits, including glutamate, serotonin, and dopamine. These neurotransmitters appear to play an essential role in the pathophysiology of OCD. It is observed that several glutamatergic neurons originating in the prefrontal cortex play a fundamental role in the CSTC circuit (Fig. 19.2) (Stein et al. 2019). Repeated stimulation of glutamatergic projections from the orbitofrontal cortex to the ventromedial striatum triggers OCD-like behaviors in mice. The authors also observed that chronic treatment with fluoxetine, an SSRI, reversed OCD-like behaviors. The authors suggest that repeated glutamatergic stimulation triggers plasticity in the CSTC circuit structures, such as the thalamus and PFC, which increases the motivational salience in the VTA. These behavioral changes from the VTA may justify the effects of fluoxetine (Ahmari et al. 2013).

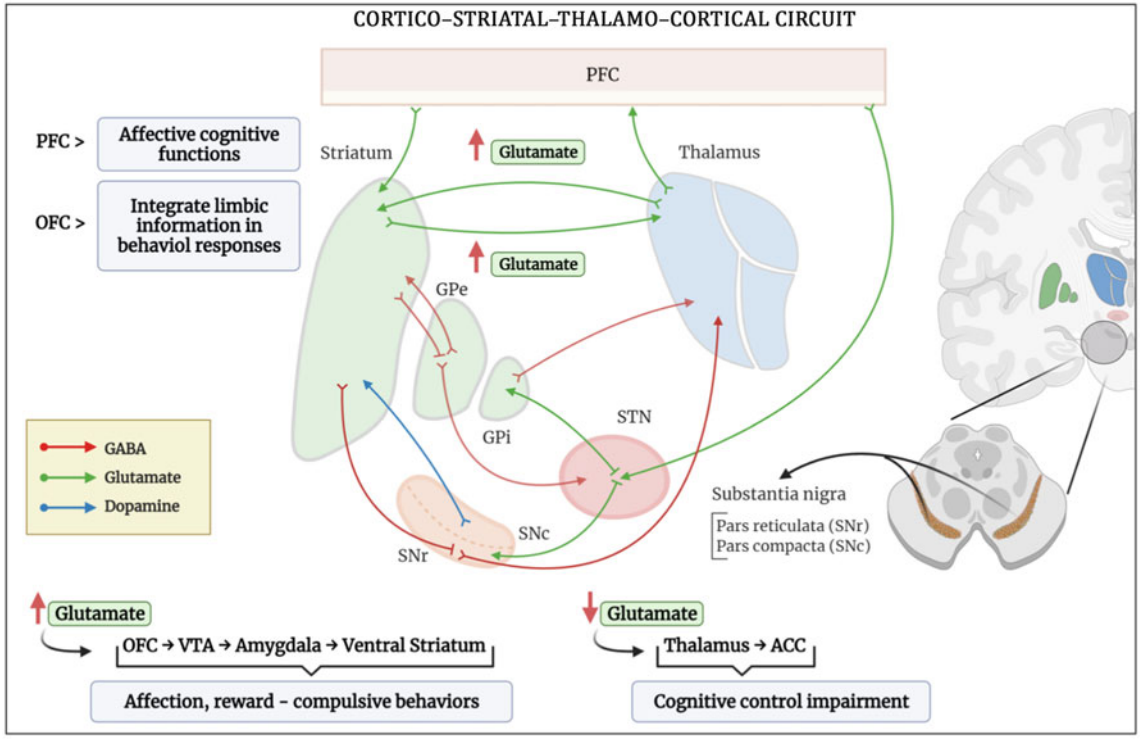

Fig. 19.2 The cortico-striatal-thalamo-cortical circuit pathway's connections with other limbic structures form a circuit whose structural and functional imbalance seems to constitute the critical mechanisms in obsessive-compulsive disorders (OCD) pathophysiology. Glutamatergic changes in the prefrontal cortex (PFC) (hyper or hypofunction) seem to trigger affective and cognitive functions in OCD. In contrast, glutamatergic hyperactivity in the orbitofrontal (OFC) seems to trigger changes in the function of the connection with the ventral tegmental area (VTA), amygdala, and ventral striatum, culminating in compulsive behaviors. A glutamatergic hypofunction between the thalamus and the anterior cingulate cortex (ACC) appears to trigger impairment in cognitive control. External and internal globus pallidus (GPe and GPi), subthalamic nucleus (STN), compact and reticular substantia nigra (SNc and SNr). Images were extracted from the BioRender app

Researchers observed a reduction in glutamatergic signaling in the mPFC and the right thalamus of unmedicated OCD using magnetic resonance spectroscopy (MRS) studies. The reduction in glutamatergic signaling in the right thalamus was significantly correlated with compulsion scores (Zhu et al. 2015). The vmPFC appears to be primarily linked to the conditioned response's expression during the retention of fear extinction learning. Damage or reduced stimulation of the vmPFC impairs the retention or recall of fear extinction (Phelps et al. 2004). In this regard, a preliminary study using MRS in OCD individuals found that glutamate levels in vmPFC were negatively associated with the recall of fear extinction and positively related to cognitive behavioral therapy (Giménez et al. 2020).

Functional connectivity at rest, defined as the temporal correlation of neuronal activation between different brain regions during rest, has been used to investigate changes in connectivity in the CSTC circuit of OCD patients. Through this approach, some studies have found an increase in functional connectivity in structures in the direct loop of the CSTC, such as the cortex, striatum, and thalamus, which points to a hyperactivation of the direct loop in OCD (Harrison et al. 2009; Fitzgerald et al. 2011). A study using functional magnetic resonance imaging (fMRI) at rest, combined with proton MRS, found functional connectivity changes in the CSTC circuit and external networks connected to the circuit. The study found in OCD patients a significant correlation between reduced functional connectivity from the right thalamus to the middle occipital gyrus. Besides, functional connectivity was negatively correlated with the total scores and the compulsion scores, measured on the Yale-Brown Obsessive-Compulsive Scale (YBOCS). The authors suggest that the increase in glutamate and reduced connectivity in this circuit may be related to affective dysfunction in OCD. In the same series of studies, it was observed that the functional connectivity between the right thalamus and the right dACC was negatively correlated with the right thalamic glutamatergic signal in OCD. The authors suggest that reducing the glutamatergic signal in this connection is related to impairment in the patients' cognitive control network (Chen et al. 2019). Another study using proton MRS found a positive correlation between glutamate in the thalamus with severity scores in OCD patients (Fan et al. 2017).

OCD patients had significantly higher resting functional connectivity between the left caudate and dlPFC, in addition to a positive correlation between caudate and dlPFC connectivity and depression scores. Greater connectivity was associated with more severe depressive symptoms (Sha et al. 2020). Since glutamate plays a critical role in the regulation of functional connectivity (Kapogiannis et al. 2013), the findings of this study suggest that changes in the caudate with increased dlPFC activity may represent the basis of the main symptoms of OCD and their relationship with depression, through excitatory transmission along the cortical-limbic-striatal circuit (Sha et al. 2020).

A study using proton magnetic resonance spectroscopy found increased relative levels of Glx and NAA in the orbitofrontal white matter of OCD patients. Higher levels of Glx/creatine were associated with the severity of symptoms. Furthermore, in the head of the caudate nucleus, myoinositol's relative values were reduced bilaterally, and such levels of myoinositol/creatine were associated with more

significant anxiety but not with the severity of OCD symptoms (Whiteside et al. 2006). A significant reduction in glutamate/glutamine (Glx) concentrations were observed in the right and left dorsal ACC in female and male OCD patients. The study also evidenced high myoinositol concentrations in the right ACC and a trend of reduction in NAA of OCD patients of both sexes. The study observed that the Glx levels in the ACC of female patients were linked to the severity of clinical symptoms and probable more lasting disorder (Yücel et al. 2008). Another magnetic resonance spectroscopy study found a significant negative correlation between myoinositol/creatine in the left orbitofrontal region and the severity of symptoms in OCD patients. Subclinical symptoms of depression and anxiety were related to metabolite rates. The authors highlight the importance of observing these psychiatric comorbidities in evaluating these metabolites in OCD individuals (Bédard and Chantal 2011).

Some researchers examined the presence of autoantibodies against the basal ganglia and thalamus and the amino acids glutamate, GABA, taurine, and glycine in serum and CSF. The researchers observed an increase in autoantibodies against the basal ganglia and the thalamus and glutamate and glycine in OCD patients. CSF glycine levels were significantly higher in OCD patients with autoantibodies. Therefore, based on these findings, it is assumed that autoantibodies do not directly cause glutamatergic abnormalities but may be involved in modulating central glycine levels. The study presented evidence that autoimmune mechanisms are present in OCD pathogenesis (Bhattacharyya et al. 2009).

The glutamatergic hyperactivity in some CSTC areas is related to the OCD pathophysiology, so some studies suggest a therapeutic potential for antagonists in Group I (mGluR1 and mGluR5). The mGluR1 receptors are expressed in cortical, limbic regions, basal ganglia, and thalamus, and their activation culminate in cellular excitability by NMDA responses. Thus, Group I antagonists can effectively balance increased glutamatergic activity in brain areas involved with OCD. Therefore, such pharmacological approaches appear to benefit OCD therapy (Spooren et al. 2003; Bhattacharyya and Chakraborty 2007).

19.7.2 Glutamate Genetic Aspects and OCD

According to the International Obsessive-Compulsive Disorder Foundation Genetics Collaborative (IOCDF-GC) and OCD Collaborative Genetics Association Studies (OCGAS) (2018), since the beginning of the last century, it is suspected that heredity plays an important role in susceptibility to OCD. One of the most recent meta-analyses that aimed to evaluate the complex genetic architecture of OCD, IOCDF-GC, and OCGAS (2018) supports some of the pre-existing findings, which point to a strong association between some genes and the OCD, for example, Slc1A1 (Solute Carrier Family 1 Member 1), excitatory amino acid transporter 3 (EAAT3) gene, Grin2B (Glutamate Ionotropic Receptor NMDA Type Subunit 2B), NR2B subunit of the NMDA glutamate receptor, Grik2 (Glutamate Ionotropic Receptor Kainate

Type Subunit 2), which encodes the subunit glutamate ionotropic receptor kainate type subunit 2, also known as glutamate ionotropic receptor 6 (GluR6), and Dlgap1, gene in the glutamatergic system that codifies disks large-associated protein 1.

Although studies to date demonstrate that the genetic etiology of OCD is complex, genes related to glutamatergic transmission are strong candidates involved in the disorder's pathophysiology. Studies have pointed to an association between Slc1A1 and the occurrence of OCD (Arnold et al. 2006; Dickel et al. 2006).

Glutamate transporters play a crucial role in finishing excitatory neurotransmission and regulating extrasynaptic glutamate levels. Thus, it has an action that limits the activation of extrasynaptic neurotransmitter receptors and consequent excitotoxicity (Danbolt 2001). In this way, the Slc1A1 gene appeared as a candidate for the OCD-related gene in the disorder's first genome-wide linkage study (Hanna et al. 2002).

The Slc1A1 gene encodes the neuronal glutamate postsynaptic transporter EAAT3, and several studies have demonstrated a possible association of Slc1A1 variants and risk for OCD (Pittenger et al. 2011; Robbins et al. 2019). One study found that a single nucleotide polymorphism (SNP), rs301443, downstream of Slc1A1, was strongly associated with OCD in the studied families and the authors suggest that the SNP is located in a region involved in the regulation of gene expression (Shugart et al. 2009). Another study from the same group confirmed the association of rs301443 and found an association of an SNP haplotype rs4740788- rs10491734-rs10491733 from Slc1A1 in family OCD (Samuels et al. 2011).

Another gene that may be related to OCD is the Sapap3 gene. This gene is responsible for encoding a critical protein to the normal localization of ionotropic glutamate receptors in the postsynaptic density (PSD) (Pittenger et al. 2011). A study of Sapap3 mutant mice identified the relevant role of Sapap3 at cortico-striatal synapses, and this study suggests that cortico-striatal synaptic defects perhaps be central to the genesis of OCD-like behaviors (Welch et al. 2007). In this way, Bienvenu et al. (2009) developed the first study to indicate the relationship between variants of the Sapap3 gene and psychiatric phenomena in humans. These researchers evidenced that variation in the Sapap3 gene is associated with human grooming disorders, which appear close and comorbid with each other and with OCD. However, variation in Sapap3 did not appear associated with OCD itself, so more studies are necessary because a study with mice has pointed out the correlation between sapap3 and OCD (Bienvenu et al. 2009).

To investigate the possible relationship between the Grik2 and ionotropic kainate 3 (Grik3) genes and the OCD, Delorme et al. (2004) investigated the frequency and transmission of glutamate receptors Grik2 and Grik3 in OCD patients. Overall, was observed a lack of association between Grik2 and Grik3 and OCD. In another study, Sampaio et al. (2011) investigated the association between polymorphisms in the Grik2 gene and OCD. As a result, the SNP rs1556995 and the haplotype of 2-markers rs1417182 and rs1556995 appeared associated with OCD. Both studies suggest the need for more research (Delorme et al. 2004; Sampaio et al. 2011).

Animal studies (Shmelkov et al. 2010) and humans (Song et al. 2017) suggest that the Slitrk5 (SLIT and NTRK like family member 5) gene may also be related to OCD. Slitrk proteins family was described by Aruga and Mikoshiba (2003), and these proteins are leucine-rich repeat-containing transmembrane proteins responsible for synapse regulation and presynaptic differentiation. Expression of the gene has been linked to the early formation of excitatory synapses. In the characterization of this gene, six different types of Slitrk proteins have been described. Among these Slitrk proteins, Slitrk5 seems to be related to OCD. According to the data, Slitrk5 may have an essential role in developing OCD-like behaviors, and the main symptoms found were self-injurious repetitive behavior and increased anxiety (Shmelkov et al. 2010). Another relevant study that indicates a relationship between the Slitrk5 gene and OCD concerns a study by (Song et al. 2017), which re-sequenced the human Slitrk gene in OCD subjects. It was observed that mutations in Slitrk 5 are significantly associated with OCD.

According to the meta-analysis (IOCDF-GC and OCGAS 2018), the Dlgap1, another gene in the glutamatergic system, is also strongly associated with OCD. Li et al. (2015) aimed to observe an association between Dlgap1 rs11081062 polymorphisms with OCD in a Chinese population. They found no association between Dlgap1 rs11081062 and OCD. However, associations were observed with OCD subphenotypes. In this way, more investigations are needed, and it appears attractive to divide OCD into more homogeneous subphenotypes (Li et al. 2015).

Despite several studies showing the relationship of critical genes in glutamatergic neurotransmission with OCD, more studies are needed to reach genome-wide statistical significance (Pittenger 2021).

19.7.3 Glutamate, Serotonin, and Dopamine Interaction in the OCD Circuit

The interaction between glutamate and the neurotransmitters serotonin and dopamine in the frontostriatal circuit seems to be an impaired functional mechanism in OCD (Fig. 19.3) (Pauls et al. 2014). A possible explanation for the therapeutic effect of IRSS is its function in the circuit, participating in the balance of glutamatergic and dopaminergic neurotransmission.

Researchers suggest that an imbalance between the direct and indirect loop paths of the CSTC circuit is a critical mechanism in the pathophysiology of OCD. Through the direct pathway, mediated by the type 1 dopamine receptor (D1), the excitatory signals from OFC and ACC increase GABA inhibitory signaling in the internal globus pallidus (GPi) and reticulate substantia nigra (SNr) (Pauls et al. 2014). Afterward, a reduction in the signal transmission triggers an increase in the thalamus's output to the cortex. In this sense, the indirect route, mediated by type 2 dopamine receptors (D2), called striatal-pallidal, acts as a modulator of glutamatergic transmission through the direct route. Also, the striatum inhibits

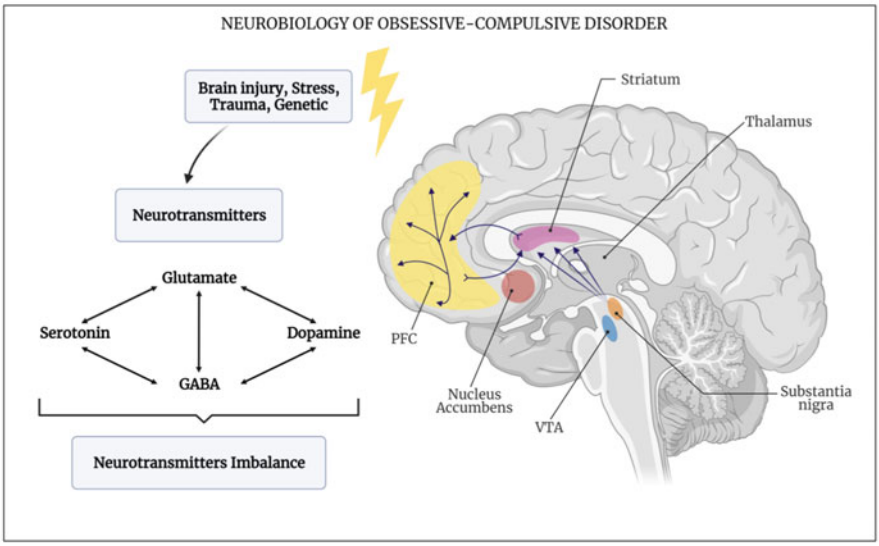

Fig. 19.3 Brain injury, life-long traumas, and stress interacting with genetic factors trigger obsessive-compulsive disorder (OCD). These factors are associated with glutamatergic changes in the cortico-limbic-striatal circuit, involving cortical areas of the prefrontal cortex (PFC), striatum, thalamus, nucleus accumbens, ventral tegmental area (VTA), among other limbic structures. Changes in the glutamatergic signal seem to involve dysregulation, mainly in serotonergic, dopaminergic, and GABAergic neurotransmission, culminating in the various behaviors inherent to OCD. Images were extracted from the BioRender app

the subthalamic nucleus (STN) and globus pallidus external (GPe), resulting in the stimulation of GPi and SNr, leading to thalamic inhibition (Vlček et al. 2018). The circuit with increased activity in the direct path disinhibits the thalamus and promotes repetitive behavioral sequences, represented by the most accepted model of OCD pathogenesis (Baxter's model) (Saxena and Rauch 2000; Ting and Feng 2008).

The D2-like receptor (D2/D3) balance in the frontostriatal circuit seems to have a relevant role in learning for sensitivity to positive and negative feedback so that low levels of D2-like receptors can infer behavioral inflexibility. Image studies in monkeys have observed that behavioral flexibility, behavioral reversal activity, and reinforcement sensitivity, which are impaired in OCD, depend on the evaluability of D2-like receptors in the dorsal striatum, influencing the indirect function of cortico-striatal connectivity (Groman et al. 2011). Changes in the frontostriatal circuit's dopaminergic modulation seem to reflect a change in the balance of serotonergic and dopaminergic activity in the dorsal striatum (Pauls et al. 2014).

The observation of the behavioral responses underlying treatment with SSRIs inspired the suggestion that serotonergic neurotransmission may reduce the release of glutamate in the PFC and striatum, thus modulating both the indirect and the

direct path, allowing a balance between the behavioral programs established by both pathways (Carlsson 2001). SSRIs are the primary drugs for treating OCD (Bokor and Anderson 2014). The OFC, striatum, and thalamus, which are part of the CSTC axis, are widely innervated by serotonergic or dopaminergic neurons (Zitterl et al. 2008). One study observed a significant reduction in the 5-HT_{2A} receptor in cortical areas of the CSTC circuit of OCD patients. The reduction of 5-HT_{2A} in OFC and dlPFC was correlated with the clinical severity of the disorder. Besides, the study results appear to point to dopaminergic hyperactivity in the striatum (Perani et al. 2008). The study did not assess glutamatergic mechanisms. However, dopaminergic changes in the striatum may likely be related to a glutamatergic function mediating serotonergic activity.

A recent study demonstrated a possible serotonin role in the pathophysiology of OCD. In this way, sertraline and cognitive behavioral therapy (CBT) can reduce OCD symptoms. However, this robust and significant effect rarely achieves complete remission, and the effect was associated with a significant increase in whole-brain 5-HT synthesis capacity in patients who respond to either treatment (Lissemore et al. 2018).

The interaction of glutamatergic neurotransmission with other key neurotransmitters in the OCD circuit still needs studies to better understand neurophysiopathology and possible treatment targets.

19.7.4 OCD and Glutamatergic Treatment

In a case study, the authors observed that memantine, an NMDA antagonist, had a therapeutic effect and reduced the severity of OCD in a patient resistant to several pharmacological therapeutic attempts. Besides, memantine was well tolerated, with few adverse effects (Poyurovsky et al. 2005). In a systematic review with a meta-analysis of eight studies involving 215 individuals, the researchers found that memantine had a positive therapeutic effect in patients with severe OCD and refractory to other therapies. The authors found that memantine was generally well tolerated and that the adverse effects were transient (Modarresi et al. 2019). However, a critical comment points to problems in Modarresi's study methodology, and that from the study, memantine cannot yet guide clinical practice. The author also concludes that memantine as an augmentation agent in OCD needs more studies (Andrade 2019).

Riluzole, a presynaptic inhibitor of glutamate release, has also been shown to improve patients' symptoms resistant to standard treatment. One study found that riluzole administered adjunctively had a positive effect on the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) symptoms. Also important, the adverse effects presented by the patients were not severe and were well tolerated (Coric et al. 2005). Adjunctive therapy with riluzole also showed a beneficial result and was well tolerated in OCD outpatients and inpatients. The study also noted a beneficial tendency for riluzole to improve symptoms of obsession in outpatients (Pittenger

et al. 2015). In treating moderate to severe OCD, riluzole adjunctive therapy to fluvoxamine improved symptoms, showing a beneficial clinical effect as a therapy of augmentation to fluvoxamine (Emamzadehfard et al. 2016).

Troriluzole, a third-generation prodrug that enhances synaptic reuptake of glutamate, was tested in phase 2/3 and showed therapeutic effect as adjunctive therapy in patients with inadequate response standard treatments. Troriluzole was well tolerated and showed a consistent safety profile, as seen in other clinical trials (Biohaven Pharmaceuticals 2020).

Regarding ketamine, a partial NMDA antagonist with a potent and rapid antidepressant effect in refractory patients, one study found a positive therapeutic effect in refractory OCD. However, the effect was better and persistent in depressive symptoms, while it did not persist in OCD after the effect of acute treatment (Bloch et al. 2012). A study of twelve untreated OCD patients evaluated the effect of ketamine infusion before oral memantine administration. Four patients treated with ketamine showed improvement in symptoms. Memantine alone had no therapeutic effect. Adjunctive memantine also does not appear to have a beneficial effect on ketamine (Rodriguez et al. 2016a). In another study, the authors observed the effect of a ketamine infusion followed by CBT and found that the acute effect of ketamine significantly reduced the severity of OCD. The therapeutic effect of acutely administered ketamine persisted with CBT therapy for 2 weeks (Rodriguez et al. 2016b).

It is also important to note a recent study, which observed that the chronic infusion of ketamine adjunctive to treatment with SSRI significantly improved the therapeutic response to standard treatments in patients with severe and refractory OCD (Sharma et al. 2020).

However, research on ketamine is still scarce and with few individuals. Some results also seem conflicting and need to be continued from further studies with protocols that detail the acute and chronic effects and possible mechanisms involved in different adjunctive therapies.

A recent study with laboratory rats found that two NMDA antagonists that target NR2A and NR2B subunits with more affinity and D-cycloserine significantly reduced anxiety-like and OCD-like behaviors, in addition to reducing glutamatergic action in the hippocampus. The NMDA antagonists and D-cycloserine reduced the NR2A and NR2B subunits levels in the hippocampus, suggesting that these drugs may be therapeutic targets for OCD by suppressing these subunits (Zhan et al. 2020). However, studies on the therapeutic function of D-cycloserine in OCD are still conflicting and scarce, requiring approaches with protocols that can specify which patients, dosage, and time for a clinical benefit (Pittenger 2021).

19.8 Conclusion and Future Directions

This review seeks to understand and discuss the research that used protocols to understand the glutamatergic function in the pathophysiology and treatment of anxiety disorders. Both study protocols in patients and animal models were

considered to understand the morphofunctional mechanisms underlying the structures of the brain circuit involved in the disorders.

The relationship between glutamate and the function of the HPA axis in stress and anxiety disorders was initially addressed, given that psychiatric disorders, in general, have stress as one of the relevant factors involved (Faye et al. 2018).

Glutamatergic neurotransmission in the PFC, which has a fundamental role in the circuit of anxiety disorders and especially in the OCD circuit, is also involved in response to acute and chronic stress, mediated by the HPA axis (Moghaddam 2002). Early life stress presents a developmental and complex interference in the HPA axis, with changes throughout life that impact the triggering and severity of anxiety disorders and OCD in adulthood (Faravelli et al. 2012). Rodents submitted to maternal separation protocols in the first days of life show anxiety and depression behaviors in adulthood, with relevant and complex changes in the HPA axis throughout life (Ignácio et al. 2017).

An important aspect that may be inherent to some conflicting results is comorbidities between the disorders. While some studies have found functional changes in the HPA axis, others have not observed these anxiety disorders and OCD (Bandelow et al. 2017; Kellner et al. 2020). One study noted that in OCD in comorbidity with depression, increased anxiety was related to increased activation of the HPA axis (Labad et al. 2018).

Most studies point to an increase in disorders due to increased glutamatergic activity regarding the role of glutamate in anxiety and OCD disorders. However, studies still need to be clarified about the interaction of glutamate with other neurotransmitters. It is essential to consider that the main treatments are still related to serotonergic, dopaminergic, and gabaergic neurotransmission in most anxiety disorders. Therefore, studies on serotonergic, gabaergic, dopaminergic, and glutamatergic neurotransmission interactions, considering the brain structures and circuits involved, are still necessary.

The evidence that glutamatergic hyperactivity is related to anxiety disorders also comes from research with compounds that reduce glutamate in neuronal endings. For example, research with patients and animal models of anxiety points to riluzole's anxiolytic effect, an inhibitor of glutamate release (Stutzmann et al. 1989; Mathew et al. 2005). A relevant factor is that riluzole's anxiolytic function appears to be related to reducing glutamatergic excitotoxicity and increasing hippocampal plasticity (Mathew et al. 2008). Another compound widely investigated for its rapid antidepressant effect, ketamine, an NMDA antagonist, has also been suggested to treat patients with anxiety refractory to other treatments (Glue et al. 2017).

Although this and other evidence suggests that glutamatergic hyperactivity may be underlying anxiety disorders, as already mentioned, research that considers glutamatergic interaction with other neurotransmitters is still scarce and needs further clarification. Some important questions come from studies with D-cycloserine, which acts on the glycine site, modulating the NMDA receptor. D-cycloserine directly in the basal amygdala nucleus seems to facilitate the learning of fear extinction, dependent on synaptic plasticity (Davis et al. 2006). Thus, D-cycloserine's function may be related to increased glutamatergic activity in

regions related to extinction learning. On the other hand, being a modulator can act moderately in some brain regions, reducing neuronal overactivation. In this line of reasoning, it is noteworthy that studies with laboratory animals, which observed an anti-OCD-like effect parallel to a reduction in the glutamatergic activity in the animals' hippocampus from D-cycloserine. The results of D-cycloserine were similar to the results of two NMDA antagonists (Zhan et al. 2020). Thus, future studies on D-cycloserine and mechanisms of action in the cortico-limbic-striated circuit may decipher some mechanisms that are still puzzling and challenging in the OCD circuit.

Many studies with important protocols on the CSTC circuit consider functional connections with limbic structures involved in regulating behaviors related to anxiety and behavioral inhibition in the OCD. Functional connections point to an imbalance of glutamatergic function in the cortico-limbic-striatal pathway. In some regions, glutamate function appears to be increased, influencing compulsive behaviors, while in others, it appears to be reduced, impairing cognitive processes of fear extinction (Zhu et al. 2015; Rasgon et al. 2017; Chen et al. 2019; Sha et al. 2020). However, studies that address glutamate's function, considering the circuit as a whole and limbic areas related to affection, fear, and behavioral inhibition, highlight glutamate hyperfunction, especially at the NMDA receptor.

OCD is a disorder that involves heterogeneous neurobiological mechanisms, which make studies complex and challenging, both to elucidate the pathophysiology and to discover more effective treatments. Besides, given the heterogeneity of mechanisms involved in the population, it is unlikely that specific treatment can induce an effective therapeutic response to all OCD patients (Pittenger 2021).

Despite some studies showing the relationship of the HPA axis with functions in the circuit, few studies focus on this relationship with glutamate. Considering that the HPA axis function can be unbalanced in many individuals subjected to stress throughout life and that stress is one of the relevant factors underlying OCD and other anxiety disorders, further studies relating the HPA axis and glutamatergic function in the circuit are necessary. They can reveal possible and relevant therapeutic targets and strategies.

Glutamatergic hyperfunction is corroborated by studies that point out the therapeutic function of NMDA receptor antagonists associated with standard therapies, such as SSRIs and CBT. However, considering that glutamatergic compounds alone do not have practical therapeutic effects, the functional mechanisms in the OCD circuit must be better understood so that the most effective therapeutic targets and strategies can be elucidated.

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Chapter 20
Modulators of Glutamatergic Signaling as Potential Treatments for Autism Spectrum Disorders

Carla Sogos and Francesca Fioriello

Abstract Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and social interaction in addition to restricted and repetitive patterns of behaviors and interests.

ASD is a lifelong condition and constitutes a major public health problem in most countries, as the rate of children being diagnosed with ASD has risen over the last two decades and researchers cannot precisely explain the reason.

Current estimates of the Centers for Disease Control and Prevention (CDC) represent a 15% increase in prevalence: to 1 in 59 children, from 1 in 68, 2 years previous.

To date, few findings are available on reliable diagnostic biomarkers and on effective treatments.

Several genetic variants of glutamatergic pathways seem to be related to ASD. In particular, Glutamate receptor, metabotropic 7 (*GRM7*), a receptor coding gene of glutamatergic pathway, is a promising candidate gene for autism.

The abnormalities of glutamatergic transmission represent potential pathophysiologic mechanisms responsible for atypical social behaviors in individuals with ASD.

The trials on glutamatergic modulators are promising but to date there is insufficient evidence to clearly support the efficacy of these drugs in ASD.

This chapter aims at evaluating the state of the art of clinical trials on glutamatergic modulators in ASD.

Keywords Autism spectrum disorder · ASD · Glutamatergic system · Glutamatergic treatments for autism · Modafinil · Riluzole · Memantine · D-Cycloserine · N-acetylcysteine · Minocycline

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Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_20

20.1 Introduction

20.1.1 History of Autism

In 1943, Leo Kanner first described early infantile autism. He noticed that: "these children have come into the world with an innate inability to form the usual, biologically provided contact with people."

Kanner (1943) identified the great heterogeneity of the disorder, but considered autism as a profound emotional disturbance without any evident cognitive deficit.

According to this description, in 1952 DSM II (American Psychiatric Association 1952) presented autism as a form of childhood schizophrenia.

Throughout the 1950s and 1960s, Bruno Bettelheim (Bettelheim 1967) introduced the view that autism was caused by negative parenting and led the way to the definition of "refrigerator mothers." These views were devastating to a generation of parents, who felt guilty and responsible for their children's disorder.

During the 1960s and 1970s, researchers and clinicians rejected the psychoanalytic theory that considered autism as the effect of a poor mother–child relationship. Autism began to be considered as a neurologic and hereditable disorder.

In 1980, the DSM III (American Psychiatric Association 1980) was published and autism was described as a "Pervasive Developmental Disorder" (PDD) clearly distinct from schizophrenia. PDD emerged before 30 months and was characterized by three specific domains: severe lack of interest in social interaction, serious deficit in communication, and atypical reactions to environment.

In 1987, DSM III was revised and published as DSM III-R (American Psychiatric Association 1987). In the revised version, the diagnosis of PDD Not Otherwise Specified was added, as a mild level of PDD.

The DSM IV (American Psychiatric Association 1994) was published in 1994 and revised in 2000. This version introduced, in addition to autism and PDD-NOS, the Asperger Disorder, the Child Disintegrative Disorder, and the Rett Syndrome. During this period research was focused on the genetic etiology of autism. Researchers hoped to find genotypes specifically correlated to different autism phenotypes.

Researchers didn't find specific genes or specific treatments for the five conditions listed in DSM IV-TR (American Psychiatric Association 2000). Based on these results, the DSM 5 (American Psychiatric Association 2013) task force proposed an all inclusive spectrum with a variety of severity levels, ranging from mild to severe. Therefore, in 2013, DSM 5 introduced the diagnosis of Autism Spectrum Disorder.

20.1.2 Autism Spectrum Disorder (ASD)

Autism spectrum disorder includes a highly heterogeneous group of neurodevelopmental disorder characterized by deficits in social communication

and social interaction in addition to inflexible, restricted, and repetitive patterns of behaviors and interests, hyper- or hypo-reactivity to sensory input, or unusual interest in sensory aspects of environment. The term spectrum highlights that ASD may present in a wide range of lifelong symptoms that may vary in form and severity.

According to DSM 5 criteria, this diagnosis includes two groups of symptoms: "persistent impairment in reciprocal social communication and social interaction" and "restricted, repetitive patterns of behavior," both present in early childhood. DSM 5 excluded Asperger syndrome, PDD-NOS, and autism, but introduced a new diagnosis of Social Communication Disorder characterized only by language and social impairments. Childhood Disintegrative Disorder and Rett syndrome were eliminated from the autism spectrum.

20.1.3 Epidemiology of ASD

Recently, the prevalence of ASD diagnosis has increased worldwide. CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network rates of ASD among children aged 8 years in the USA have increased from approximately one in 150 children during 2000–2002 to one in 68 during 2010–2012 (Baio et al. 2018a, b). Based on the latest results (2018) of ADDM, about 1 in 59 children in the USA was diagnosed with ASD. In other studies, rates vary from 1 in 67 (Lyall et al. 2017) to 1 in 132 (Baxter et al. 2015). According to Fonbonne (2009), this variability is presumably due to different methodological approaches across countries. Several researchers hypothesized that the definition of autism was too narrow in the past and that the recently observed increased rates of ASD may be a consequence of changes in public awareness and in the interpretation of diagnostic criteria, especially as they apply at milder severity levels.

ASD occurs more frequently in males, with a male to female ratio of around 4:1. This led to considering autism as a male-dominated diagnosis. In the last years, many papers questioned whether this difference in prevalence could come from underdiagnosis of ASD in females. Autism may have a different and possibly more subtle presentation in girls, so prevalence in this sex could be higher than what appears in the data at the moment (Dworzynski et al. 2012; Zwaigenbaum et al. 2013; Wickens et al. 2018).

There is an important association with epilepsy and epileptiform or non-epileptiform EEG abnormalities and autism. This may be determined by structural alterations, metabolic dysfunctions, or genetic defects with a role in etiopathogenesis in both disorders (Keller et al. 2017).

20.1.4 Early Diagnosis of ASD

ASD is still frequently diagnosed after the age of 4 years, despite the fact that autistic signs appear very early in life and that early intervention may significantly improve the outcomes of children with ASD.

No specific effective treatments are available for ASD. Since several researches evidence that an early rehabilitative intervention may improve the prognosis of the disorder influencing the development of neural pathways, early diagnosis becomes crucial. Given how complex the brain is, it can be very difficult to modify difficulties in brain development. This is why treatment for autism needs to be so intensive, and why early diagnosis and treatment are so important.

Symptoms of autism typically become evident during the first three years of life. Some children show atypical signs from birth; others show symptoms later at 18–-36 months old. However, it is now well known that some patients may not show symptoms of a communication disorder until demands of the environment exceed their capabilities.

20.1.5 Genetics and Risk Factors

Given the increasing rates of ASD, the research of diagnostic markers has gained considerable attention. ASD represents a high social cost, more than heart and cancer disorders associated. Therefore there is a pressing need to identify specific ASD biomarker in order to improve the reliability of symptomatic diagnosis.

The pathophysiology behind autism is still unknown, and no single etiological and pathophysiological mechanism is involved. There are many different etiologies behind autism spectrum disorder (Fig. 20.1).

Researchers agree that ASD has a strong genetic component and a high heritability. However, the recent studies in genetic of ASD evidence a considerable genetic heterogeneity and a lack of specific transmission model or major gene causing the disorder.

Numerous genes have been implicated and in most of the ASD cases there are many mutations, but each of these seems to only have a small effect. Most commonly, the mutations are copy number variants and often occur de novo, meaning that they are not present in the mother and father's DNA, partly explaining why ASD can occur in families with no previous cases.

ASD can be syndromic or non-syndromic. Around 10% of the cases are syndromic, meaning they are associated with single gene defects and chromosomal abnormalities. The most common gene defect is fragile X syndrome (FXS), present in 2–3% of ASD cases, caused by a mutation in the FMR1 gene and causes learning disabilities and moderate to severe cognitive impairment. Another Mendelian

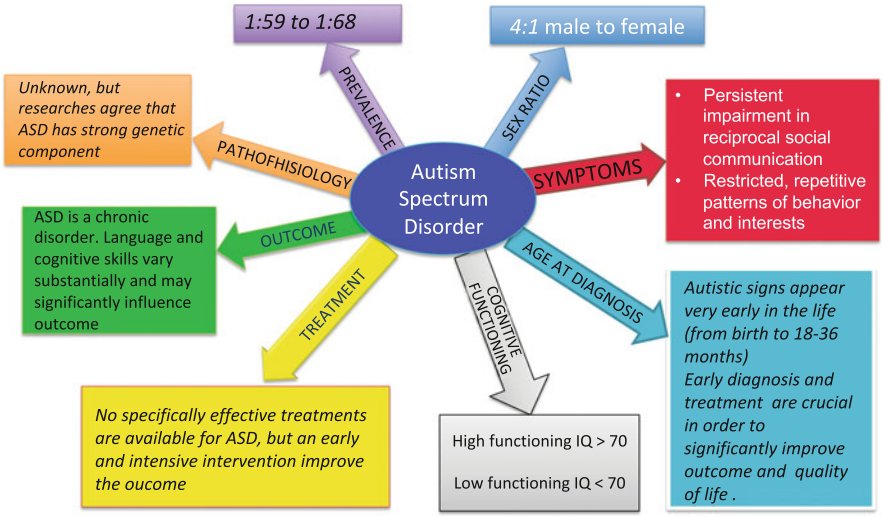

Fig. 20.1 Autism spectrum disorder

condition associated with autism is Tuberous Sclerosis Complex, with alterations found on genes TSC1 and TSC2 encoding hamartin and tuberin, respectively. Many chromosomal abnormalities have been described in ASD, such as rearrangements in the region 15q11-15q13 of chromosome 15, regions 7q22 and 7q31 of chromosome 7, deletions in 2q37 of chromosome 2.

It is expected that increasing genetic knowledge will raise the percentage of specific genotypes and phenotypes in individuals with ASD. Regarding heritability of ASD, it is worth noting that almost 20% of younger siblings of a child with ASD will meet a diagnosis of ASD. This frequency is much higher than the 1–2% risk among the general population reported by CDC.

Actually, a growing body of research focuses on the underlying objective, effective, and specific biological mechanisms and the potential pathways connecting genetic evidences and non-genetic factors in the etiology and pathogenesis of ASD.

Potential environmental risk factors are pollution, valproate exposure, advanced parental age, pregnancy-related complications and medication use, and maternal smoking (Bölte et al. 2019).

New evidences support the hypothesis that multiple genetic and environmental risk factors for ASD contribute to disrupt the balance between glutamate-mediated excitatory and gamma-aminobutyric acid (GABA)-mediated inhibitory neurotransmission. The disruption of excitatory-inhibitory balance may address treatment targets for the disorder (Nelson and Valakh 2015).

20.2 Glutamatergic System in ASD

20.2.1 Glutamatergic Physiology

Glutamate is the principal excitatory neurotransmitter in the brain. It is essential for normal brain development and plasticity and is generated from glutamine in the presynaptic terminals of neurons by the enzyme glutaminase.

Glutamate modulates excitatory neurotransmission via different types of ionotropic and metabotropic receptors. Ionotropic glutamate receptors are involved in fast excitatory neurotransmission, whereas Group I metabotropic receptors are specific for slower excitatory neurotransmission and Group II and Group III metabotropic receptors are responsible for inhibitory neurotransmission.

The three ionotropic glutamate receptors are: N-methyl-D-aspartate (NMDA), 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA), and kainate; all named for specific agonists that bind the receptor with high selectivity. All NMDA receptors (NMDARs) and AMPA receptors (AMPARs) consist of four subunits, with peculiar functions. These receptor subtypes were renamed on the basis of their subunit composition. AMPA receptors may be calcium-permeable or calcium-impermeable, based on the absence or presence of the GluA2 subunit (Hanley 2014). NMDA receptors constitute two GluN1 and two GluN2 (or GluN3 subunits). The four subtypes of GluN2 subunits (GluN2A-2D) determine the functional difference and specific biophysical and pharmacological properties (Wickens et al. 2018).

The eight metabotropic glutamate receptors (mGluR) are a class of G-protein coupled receptors and are simply identified as 1–8. They are classified into three groups based on receptor structure, functional similarity, and common agonists (Niswender and Conn 2010).

Group I receptors consisting of mGlu1 and mGlu5 mainly potentiate presynaptic glutamate release and interfere with ionotropic NMDAR receptor.

Group II consisting of mGlu2 and mGlu3 receptors mediate glutamate release, mainly during the synaptic transmission.

Group III receptors consisting of mGlu4, mGlu6, mGlu7, and mGlu8 receptors inhibit glutamate function.

Considering the complexity of glutamatergic system, an efficient glutamatergic neurotransmission depends on the dynamic interactions among glutamate receptors and other molecular components such as adhesion proteins, neuroligins, scaffolding proteins, vesicle proteins, and transporters (Table 20.1).

20.2.2 Glutamatergic Receptors and ASD

Group 1 mGluRs including mGlu1 and mGlu5 and Group III mGlu7 seem to be specifically involved in ASD and in some other neurodevelopmental disorders. In

Table 20.1 Glutamatergic physiology

Glutamate	Excitatory neurotrasmitter
Receptors	Ionotropic and metabotropic
Functions	*Ionotropic receptors* are involved in fast excitatory neurotransmission; *group I metabotropic* receptors are involved in slower excitatory neurotransmission, *group II and group III* metabotropic receptors are involved in inhibitory neurotransmission
Ionotropic receptors	N-methyl-D-aspartate (*NMDA*), 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (*AMPA*), and kainate
Metabotropic receptors	mGluR1, mGluR2, mGluR3, mGluR4, mGluR5, mGluR6, mGluR7, mGluR8. They are classified into 3 groups based on receptor structure, functional similarity, and common agonists

particular, mGlu7 is considered the most promising candidate for identifying therapeutic agents for ASD. Actually mGlu7 is the most widely expressed mGlu receptor in the central nervous system (Kinoshita et al. 1998). Preclinical studies evidenced that reduced mGlu7 levels may correlate to aspecific symptoms of neurodevelopmental disorders. The same symptoms may improve in rodents through positive modulation of mGlu7. Further researches are necessary to better understand which subtypes of NDD may benefit from an mGlu7-mediated therapy (Fisher et al. 2018).

Even glutamatergic signaling plays an important role in the pathology of ASD particularly through N-methyl-D-aspartic acid (NMDA) receptor (Uzunova et al. 2014). This receptor is stimulated by glutamate when specific D-serine or glycine coagonists occupy its allosteric site. Glycine has a high affinity for extrasynaptic NMDARs. D-serine has a high affinity for synaptic NMDARs.

Several mutations or allelic variants, that may interact with brain development and behavior, have been evidenced in autism. Furthermore, Bioinformatic analyses of 99 modified genes were associated with human autism. Gene expression patterns in preclinical studies show significant enrichment in autism-associated genes and the NMDA receptor gene family was identified among these. Recently, the monoclonal antibody-derived tetrapeptide GLYX-13 was evidenced to function as an N-methyl-D-aspartate receptor modulator and it shows the possibility to cross the blood–brain barrier. Treatment with the NMDAR glycine site partial agonist GLYX-13 solved the deficit in the animal model. Seems to play a functional role in autism, and GLYX-13 become suggestive for the treatment of autistic individuals (Santini et al. 2014).

20.2.3 Increased Serum and Blood Glutamate in ASD

Several studies have demonstrated elevated serum glutamate in autism subjects (Moreno-Fuenmayor et al. 1996; Shimmura et al. 2011; Tirouvanziam et al. 2012).

Only Rolf et al. (1993) described lower glutamate levels in ASD.

Given that glutamate does not go through the blood–brain barrier, it is still unclear if these results depend on CNS amino acid levels.

Furthermore, in postmortem brain tissue of ASD subjects, Shimmura et al. (2013) found elevated levels of glutamate and glutamine from the anterior cingulate cortex.

20.2.4 Hyperglutamate Vs Hypoglutamate Theory of ASD

Glutamate abnormality in blood plasma and serum has been proposed as a central factor in the pathogenesis of ASD. In 1998, Carlsson proposed that infantile autism could be a hypoglutamatergic disorder and hypothesized possible pharmacological interventions based on the strict interaction between central glutamate and serotonin, notably the serotonin (5-HT) 2A receptor (Carlsson 1998).

On the reverse side, in 2008 Fatemi proposed the hyperglutamate theory of autism based on several studies that showed elevated levels of glutamate in blood samples of children and adults with ASD.

20.2.5 Altered Glutamate–Glutamine Balance in ASD

The enzyme glutaminase catalyzes the synthesis of Glutamate from glutamine. A great amount of the extracellular glutamate is reabsorbed by astrocytes and converted back into glutamine. Glutamine may be stored in astrocytes or transformed to Glutamate in both glutamatergic and GABAergic neurons (Rowley et al. 2012). This process is identified as the glutamate-glutamine cycle and it is necessary to maintain the constant flux of GABA, glutamine, and Glutamate. In ASD, alteration of enzymes controlling glutamate-glutamine cycle has been found (Fatemi et al. 2011; Shimmura et al. 2013; Yip et al. 2007; Brondino et al. 2016) and consequently atypical metabolism in ASD.

Some studies described different alterations of glutamate and glutamine levels in children with autism despite the fact that levels of glutamate should be highly predictive of glutamine levels. A study (Shimmura et al. 2011) described higher levels of glutamate and lower glutamine levels in children with ASD compared to controls.

It is worth mentioning that glutamate is synthesized even into GABA by glutamic acid decarboxylase (GAD). There has been described two GAD isoforms: GAD 67 and GAD 65 that produce GABA for specific functions within the neuron. Several researches have described reduced GAD expression in postmortem tissue of individuals with autism. Other studies have reported lower GAD65 and GAD67 in cerebellar and parietal cortex tissues from individuals with ASD compared with control samples. Reduced GAD expression in ASD seems to be related to increased Glutamate concentration and also with decreased level of GABA (Gogolla et al.

2009; Harada et al. 2010; Gaetz et al. 2013). The studies of these alterations in the balance between glutamate-mediated excitatory and GABA-mediated inhibitory neurotransmission may potentially allowed a better comprehension of etiology of ASD and may address possible future treatments (Nelson and Valakh 2015).

Decreased GABA levels in the somatosensory cortex seem to be interlinked with atypical tactile function in ASD children (Puts et al. 2017). It is worth noting that in preclinical studies, atypical tactile function is connected to ASD social deficits.

Ajram et al. (2019) in an accurate review of recent studies in pediatric populations (Brix et al. 2015; Cavalho Pereira et al. 2018; Drenthen et al. 2016; Gaetz et al. 2014; Goji et al. 2017; Harada et al. 2011; Ito et al. 2017; Kubas et al. 2012; Puts et al. 2017; Rojas 2014; Horder et al. 2011) reassumed that the alteration of excitatory-inhibitory system could be considered as a promising biomarker for ASD.

Actually, using [1H] MRS, Ajram et al. (2017) found a different E-I responsivity between ASD and control subjects and may mean that the same treatment may have different effects in ASD compared to typically developing individuals. Furthermore this difference may explain the heterogeneous responses to pharmacological treatments in ASD individuals and some paradoxical effects that some ASD may present to different medications.

20.2.6 Glutamatergic Pathways and ASD

It is well known that a central network in the pathology of psychiatric disorders is represented by glutamatergic signaling, through N-methyl-D-aspartic acid (NMDA) receptor. This receptor is activated by glutamate when specific D-serine or glycine coagonists occupy its allosteric site. Glycine is considered the main coagonist in the spinal cord and in the hindbrain, and it has a high affinity for extrasynaptic NMDARs. D-serine is the main coagonist in the forebrain.

Glutamate receptor, metabotropic 7 (*GRM7*), a receptor coding gene of glutamatergic pathway, is a promising candidate gene for autism (Noroozi et al. 2016).

To date, in vivo molecular imaging researches on glutamate receptors in ASD are insufficient.

Using postmortem evidences, the glutamate receptor densities and protein levels of subjects with ASD were compared with samples of neurotypical individuals.

The density of AMPA-type glutamate receptor was found to be reduced in the cerebellum of individuals with ASD (Purcell et al. 2001). The same authors reported increased density of the glutamate transporters EAAT1 and EAAT2 in the post mortem cerebellum of ASD individuals.

Furthermore, increased levels of metabotropic glutamate receptor 5 (mGluR5) were described in the vermis of children with ASD (Fatemi 2008).

20.2.7 Researches in Living Human Brain

Different results have been acquired in vivo. Many studies have measured the levels of both glutamate and glutamine with proton magnetic resonance spectroscopy (MRS) in humans.

Page et al. (2006) using MRS reported higher levels of glutamate in the right hippocampus in ASD individuals compared to a group of typically developing subjects. A different area in the parietal cortex did not show the same results.

Other researches reported high glutamate levels in several different regions (Joshi: 2012ir; Bejjani et al. 2012; Brown et al. 2013), but, on the contrary, other studies evidenced lower level of Glutamine, glutamate, and GABA or similar level of glutamina, glutamate, and GABA in autism subjects compared to different groups (Bernardi et al. 2011; Horder et al. 2013).

Actually, opposite findings have been reported on these assumptions.

Contrasting results have been described on single gene disorders associated with autism using proton magnetic resonance.

Bruno et al. (2013) described decreased level of Glutamate, glutamine, and GABA in the caudate nucleus of patients with fragile X syndrome. The reverse has also been described by Pan et al. (1999) who found elevated gray matter concentration of glutamate in patients with Rett syndrome.

No specific studies of the glutamate system have been carried out with either position emission tomography (PET) or single-photon emission computed tomography (SPECT) in ASD individuals.

It is worth noting that results of in vivo studies may vary based on the age group sampled. Actually, ASD is a neurodevelopmental disorder and the results depend on brain maturation.

Despite these limitations in the last years many encouraging [I] MRS studies of excitatory-inhibitory balance in pediatric population of ASD have been conducted (Ajram et al. 2019).

20.2.8 Genetics and Glutamate in Autism

Recently, Tick et al. (2016) reported the results of a meta-analysis of ASD twin studies defining the sheritability of ASD in a range from 64 to 91%. They hypothesized that presumably the variability in ASD between different countries is the result of genetic differences. Autism has a well-known genetic base, nevertheless in most cases it is still unclear a specific etiology. Betancur (2011) described a genetic etiology in about 10–20% of cases of ASD. The most frequent was the fragile X syndrome caused by a mutation in the FMR1 gene. Furthermore, Sebat et al. (2007) found another 10% of ASD with copy number variations.

More recent studies reported in ASD subjects a possible association of excitatory neurotransmission alteration with genes coding for cell-adhesion proteins. Vaags

et al. (2011) described deletions of presynaptic Neurexin 1 (NRXN1) and NRXN3 connected to autism spectrum disorder. Variations of post synaptic Neuroligin 3 (NRLGN3) and 4 (NRLGN4) have been considered rarely associated with autism (Jamain et al. 2003; Laumonnier et al. 2004; Talebizadeh et al. 2006).

The Autism Genome Project Consortium et al. (2007); Jacob et al. (2011) described a possible link between ASD and the glutamate transporter genes SLCIA1 and SLCIA2. Several studies confirmed a strong genetic base on glutamate receptor's and transporter's alterations in ASD individuals.

Genetic association between Autism Spectrum Disorders and GRM7, the gene that codes for mGlu7 in humans has been studied in several preclinical researches. Gai et al. (2012) and Liu et al. (2015) found heterozygous deletions in GRM7 in 4 ASD patients. Yang and Pan (2013) described two specific polymorphisms: the SNP rs6782011 and rs779867 that showed a significant association with ASD in a group of 22 ASD patients. In an Iranian cohort of 518 ASD individuals, an SNP rs779867 that associates ASD with GRM was reported by Noroozi (2016).

20.2.9 Sex Differences in Glutamate System in ASD

As above reported, male and females with ASD exhibited differences in prevalence and presumably in symptoms. To gain a better understanding of sex differences in ASD and identifying possible sex variations in treatment effectiveness, it is necessary to study in depth potential sex differences in glutamate system.

Wickens et al. (2018) in an accurate review on this topic described sex differences in many aspects of Glutamate system and considered how much more work is necessary for a deeply understanding of how sex peculiarities in glutamate may interact with the other components of the disease.

Studies using proton magnetic resonance spectroscopy have shown a slight increase in glutamate levels in the parietal gray matter of men compared to women (Sailasuta et al. 2008).

On the opposite, Zahr et al. (2013) described higher levels of glutamate in women compared to men in the striatum and cerebellum. Sex differences of glutamate concentrations were also observed in blood (Zlotnik et al. 2011) and serum (Shulman et al. 2006). Notably, sex differences in glutamate concentrations change across the lifespan. Sex differences were described in glutamate level and in glutamate receptors as well. Results showed a slightly sex difference in glutamate transmission in young adulthood but this difference during age is amplified and may explain different sex differences in prevalence, symptoms, and treatment efficacy.

As reported above, boys are four times more likely to be diagnosed with ASD than girls (Fonbonne 2009; Elsabbagh et al. 2012).

Several studies have demonstrated that in ASD individuals reduced concentration of glutamate metabolites in the basal ganglia and in the anterior cingulate cortex is correlated with severity of autistic disorder (Horder et al. 2013; Tebartz van Elst et al. 2014).

Furthermore in children with ASD, symptom severity appears to be correlated with increased concentration of glutamate in plasma (Cai et al. 2016). Notably, no researches have examined sex differences in glutamate concentration despite the well-known sex differences in prevalence of ASD.

20.2.10 Single-Gene Conditions and Glutamate in ASD

The most known single gene conditions associated with ASD are fragile X syndrome, tuberous sclerosis, and 22q13 deletion syndrome.

Fragile X syndrome, presenting in males approximately in 1 in 3600–4000 subjects and in females in 1 in 4000 to 6000, is caused by a single gene mutation affecting fragile X mental retardation protein (FRMP) (Oostra and Verkerk 1992). The data of the Centers for Disease Control and Prevention show 46% of males and 16% of females with FXS have been diagnosed or treated for ASD.

These conditions and in particular the advances in the knowledge of fragile X syndrome contribute to a better understanding of glutamate alterations in ASD individuals and might lead the way to new possibilities of treatment.

20.3 Current Treatment and Modulators of Glutamatergic System for ASD

The abnormalities of glutamatergic transmission represent potential pathophysiologic mechanisms responsible for atypical social behaviors in individuals with ASD. Glutamate, the main excitatory neurotransmitter in the brain, acts a central role in cortical development and plasticity (Manent and Represa 2007). Glutamatergic dysfunction seems to be central in etiopathogenesis of ASD. Actually several studies have described atypical peripheral glutamate concentrations, altered glutamate expression in the postmortem brain tissue, and genetic atypia in glutamate signaling genes in subjects with ASD (Johnston 1995). In fact, concentration of glutamate decarboxylase, the enzyme deputed to conversion of glutamate into γ-aminobutyric acid (GABA), was evidenced to be lower in the postmortem brain tissue of patients with ASD (Fatemi et al. 2011). Concentrations of the glutamatergic *N*-methyl-d-aspartate (NMDA) receptor, which is indispensable for learning and memory, have been shown to be high in the postmortem brain tissue of individuals diagnosed with ASD (Purcell et al. 2001). High levels of glutamate during brain development result in formation of defective neural pathways and negatively affect higher cortical functions (Johnston 1995). The effectiveness of glutamate-modulating drug on autistic disorders further supports the role of glutamate in the pathophysiology of this disorder.

In this chapter we described the six more promising glutamatergic agents in ASD.

20.3.1 Minocycline

Minocycline ((4S, 4AS, 5ar,12as)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide) is a second generation tetracycline antibiotic agent that easily crosses the blood–brain barrier. This drug acts bacteriostatically against a broad spectrum of germs; furthermore, it seems to have beneficial effects on anti-inflammation of the central nervous system, on microglial activation, and on neuroprotection. In several studies, minocycline has shown promising results in patients with psychological disorders, for example depression (Miyaoka et al. 2012) and schizophrenia (Khodaie-Ardakani et al. 2014). Recent research found minocycline to reduce glutamate excitotoxicity and augment neurogenesis (Dean et al. 2011). It has a proven tolerability and safety profile for clinical use.

Some studies have shown that minocycline has properties that influence glutamatergic pathways, in particular, following glutamate administration, minocycline-treated rat cortical neurons had increased cell viability compared with controls, indicating decreased glutamate-induced neurotoxicity (Kraus et al. 2005; Morimoto et al. 2005).

Several studies about neurophatic pain have shown that glutamate deficient intake by glial cells into spinal sensory synapses resulted in increased activation of the NMDA receptor; in rats treated with minocycline, emerged a reduced absorption of glial glutamate in the spinal sensory synapses (Nie et al. 2010).

This drug can be an interesting candidate in management of ASD by inhibiting the probable underlying neuroinflammatory mechanism and its immunomodulatory characteristics.

Another possible minocycline mechanism of action in ASD could be through reduction of metalloproteinase-9 (MMP-9); it is a zinc metalloproteinase enzyme that is responsible for extracellular matrix degeneration and it is involved in synaptic plasticity and learning processes (Ganguly et al. 2013). Furthermore, increased levels of MMP-9 are known to be associated with neuroinflammation and neurodegeneration (Kaplan et al. 2014). One study showed an increased level of MMP-9 in patients with ASD (Abdallah et al. 2012).

Another possible role of minocycline could be in the regulating N-methyl-D-aspartate (NMDA) receptors. Several mutations in genes encoding subunits of NMDA receptors, in particular de novo, have been reported in patients with ASD (Kenny et al. 2014), which are suspected to alter the function of these receptors and NMDA receptor-dependent plasticity (Lee et al. 2015).

Recently, Ghaleiha et al. (2016) investigated, in a randomized, double-blind placebo-controlled trial, the efficacy of minocycline as an add-on therapy to risperidone in reducing severity of ASD symptoms. 46 children, within the age range of 4–12 years, were selected and randomized to receive minocycline plus risperidone or placebo plus risperidone for 10 weeks. Patients were evaluated using Aberrant Behavior Checklist-Community (ABC-C). Minocycline improved patients' symptoms in subscales of irritability and hyperactivity/noncompliance, while no

improvements were observed in subscales of lethargy/social withdrawal, stereotypic behavior, and inappropriate speech.

No serious adverse events were observed in the two groups.

Another study by Pardo et al. (2013) investigated the efficacy of minocycline in an open-label trial on 10 children with ASD of the regressive subtype. Patients took minocycline for six months. Cerebrospinal fluid (CSF), serum, and plasma were obtained before and at the end of treatment and were analyzed for markers of neuroinflammation; furthermore, behavioral measures were collected.

The authors observed only minimal clinical improvements in some of the patients. There were no significant changes in the quantitative assessment of cytokines and chemokine in serum and in cerebrospinal fluid, with the exception of a reduction in the levels of CXCL8 in serum. BDNF (the truncated-BDNF form) in CSF showed a significantly lower concentration post-treatment, instead the hepatic growth factor (HGF) was found to be significantly increased in the CSF after treatment. These results suggest that minocycline might have effects in the CNS by modulating the production of neurotrophic growth factors.

Adverse events reported included gastrointestinal and upper respiratory symptoms. Side effects were reported in three subjects (benign hematuria, weight gain, pica, teeth staining, increased aggression, and head-banging).

20.3.2 Riluzole

Riluzole (2-amino-6-trifluoromethoxy benzothiazole) is a benzothiazole with anxiolytic, neuroprotective, anticonvulsant, and anesthetic properties (Kretschmer et al. 1998). Its effects are mediated by block of glutamate transmission, the stabilization of sodium channels and blockage of gamma-aminobutyric acid reuptake (Mechler et al. 2018).

Riluzole acts on glutamate transmission, it uses extracellular glutamate through several complex processes. This drug inhibits glutamate production, reduces glutamic acid release, and enhances glutamate synaptic uptake.

At the presynaptic nerve cell terminal, riluzole inhibits the release of glutamate and enhances glutamate reuptake. This drug also interferes with postsynaptic effects of glutamate by noncompetitive blockade of alfa-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors. The efficacy of AMPA receptor modulators has been established in many psychiatric and neurological diseases; altered density of AMPA receptors has also been demonstrated in patients with ASD (Ghaleiha et al. 2013).

Furthermore, riluzole is thought to practice neuroprotective effects by potentiating gamma-aminobutyric acid type A (GABA A) neurotransmission.

This drug is generally well tolerated in the pediatric populations.

Recently, Wink et al. 2018 investigated, in a randomized, double-blind placebo-controlled trial, the safety and tolerability of 5 weeks of adjunctive riluzole treatment for drug-refractory (to the first-line treatment) irritability in 7 patients with autism

spectrum disorder, within the age range of 12–25 years. All participants were treated with at least one concomitant antipsychotic during the study and many were taking additional behavioral medications.

Riluzole was well tolerated by study participants, in fact there were no clinically significant laboratory abnormalities, blood pressure and heart rate remained stable for study participants. However, no significant treatment effect was identified on the target symptoms.

The results of this study must be interpreted in the context of its limitations, in fact this study enrolled only 7 patients with ASD and ongoing irritability previously unresponsive to drug treatment. It's important to underline that this drug was well tolerated by all participants.

Another study by Nicolson et al. (2017) investigated the safety and efficacy of riluzole in treating the core symptom domains in ASD. 54 patients with ASD, within the age range of 8–14 years, participated in a randomized, double-blind placebo-controlled trial of riluzole. The drug was well tolerated, without serious adverse events reported by participants. The riluzole was not superior to placebo in terms of reduction in the core symptom domains of ASD, in fact there were no significant differences between the two groups with regard to social withdrawal, repetitive behavior, or ritualistic behavior.

However, patients taking riluzole did have a significantly greater and clinically meaningful reduction in their score on the Aberrant Behavior Checklist-irritability and Aberrant Behavior Checklist-Hyperactivity subscales; both of which are interfering symptoms commonly associated with ASD.

In another study, Ghaleiha et al. (2013) investigated, in a randomized, double-blind, parallel-group, placebo-controlled trial, the tolerability and efficacy of riluzole as an add-on therapy to risperidone in reducing irritability in 40 children with ASD, who were not optimally responding to previous treatments. Children are between 5 and 12 years old. The patients received riluzole or placebo in addition to risperidone for 10 weeks.

Patients in the riluzole group showed a significantly greater improvement on the Aberrant Behavior Checklist-Community subscales "irritability, lethargy/social withdrawal, stereotypic behavior, and hyperactivity/noncompliance." Interestingly, 55% of children in the active treatment group versus 25% in the placebo group were classified as responders based on their CGI-I scores.

Children in the riluzole group experienced significantly more increases in their appetite and bodyweight than children in the placebo group by the end of the study, there was no significant difference in the frequency of other side effects between the two groups.

20.3.3 Modafinil

Modafinil ((+/−)-2-(benzhydrylsulfinyl)acetamide) is a psychostimulant which works indirectly on the glutamate and GABA receptors. This drug stimulates the

release of glutamate in both the hippocampus and the thalamus, although its precise mechanism of action remains unclear (Gerrard and Malcolm 2007). Additional indirect modulation of neurotransmission includes an increase in dopamine, noradrenaline, and serotonin secretion. In the literature there are no studies available on the efficacy and safety of this drug in patients with autism spectrum disorder. Modafinil is mainly used as a waking agent for sleeping disorders. Further uses of this drug include treatment for attention deficit/hyperactivity disorder, depression, and depressive episodes in bipolar disorder (Patin and Hurlemann 2015).

20.3.4 Memantine

Memantine (3,5-dimenthyladamantan-1-amine) antagonizes the action of glutamate and its receptors, most likely mediated principally through the voltage-dependent blockade of current flow through N-methyl-D-aspartate (NMDA) receptor channels (Mechler et al. 2018; Johnson and Kotermanski 2006; Parsons et al. 2007).

Recently, Hardan et al. 2019 investigated, in a three phase 2 trials, the efficacy and long-term safety of weight-based memantine extended release treatment in children with autism spectrum disorder. All participants were children aged 6–12 years. All studies have used the social responsiveness scale (SRS), the children's communication checklist second edition (CCC-2), the clinical global impression (CGI), and aberrant behavior checklist-community version (ABC-C).

The first study, a 50-week open-label trial, identified memantine extended-release treatment responders for enrollment in second study and assessed safety and tolerability of this drug. 765 children completed this trial. The responsive criterion was defined as an equal to or more than ten-point improvement on SRS total raw score from baseline, about 75% of all sample achieved improvement in SRS total raw score.

The side effects were: headache, nasopharyngitis, pyrexia, and irritability. Serious adverse events occurred in 6 children: abnormal behavior, accidental exposure, constipation, disinhibition, and gastroenteritis.

Participants who completed equal to or more than 12 weeks of treatment and met the defined responsive criterion at two consecutive visits separated by at least 2 weeks were eligible to transition to randomized trial (second study).

The second study was a 12-week randomized, double-blind, placebo-controlled, withdrawal. The purposes of this study were to evaluate the safety, tolerability, and efficacy of memantine extended release versus placebo in patients previously on stable memantine therapy. 479 children completed the study. At week 12, no clinically meaningful changes from baseline were observed between treatment groups on the additional efficacy variables, clinical global impression-improvement (CGI-I) scale and clinical global impression-severity (CGI-S) scale, ABC-C, or SRS subscales, and SRS total raw score. The percentages of participants with side effects were similar across treatment groups, the most common were irritability, vomiting, agitation, and anxiety. Most side effects were mild to moderate in intensity. A total

of six participants reported severe side effects: two with memantine extended release and four with placebo.

The last study was an open-label extension trial in which participants from the other two studies were treated for less than or equal to 48 weeks with memantine extended release. The purpose of this study was to assess the long-term safety and tolerability of memantine extended release. 81 children completed the study. About half of the sample reported at least one side effect; the most common side effects reported were nasopharyngitis, vomiting, pyrexia, and headache. A total of 17 participants discontinued due to an adverse effect: namely aggression, abnormal behavior, anxiety, irritability, and increased weight. By the end of this study, there was a mean $\pm$ SD (Standard Deviation) decrease (improvement) in SRS total raw score of 32.4 ± 26.4 from baseline of the first lead-in study.

In another study Aman et al. (2017) investigated, in a randomized placebo-controlled 12-week trial and 48-week open-label extension, the safety, tolerability, and efficacy of memantine extended-release. 104 children completed the first study, 66 children completed the second study, within the age range of 6–12 years.

The first study was a 12-week, randomized, double-blind, placebo-controlled, parallel-group, this study investigated the safety, tolerability, and efficacy of memantine extended-release in children with autism. Analysis of the change from baseline in SRS total raw score at week 12 showed no statistically significant difference between the treatment groups; however, both groups achieved a clinically significant improvement from the baseline. There were no statistically significant difference between groups in core autism treatment scale-improvement (CATS-I), but both groups demonstrated overall improvement versus baseline.

All side effects were mild or moderate in severity except for three: irritability, affective disorder, and choking.

The second study was a 48-week, multicenter trial that evaluated the long-term safety and tolerability of memantine extended-release in the same children of the first study. All participants showed an improvement in SRS total raw score and in CATS-I.

No clinically significant changes occurred in clinical laboratory values, vital signs, or electrocardiogram.

These studies did not demonstrate clinical efficacy of memantine extended release in autism; however, the tolerability and safety data were reassuring.

In another study Nikvarz et al. (2017) investigated, in a randomized open-label trial, the efficacy of memantine versus efficacy of risperidone in reducing severity of ASD symptoms, in particular stereotyped behaviors, impairment in social interactions, and communication skills. 30 children, within the age range of 4–17 years, were selected and randomized to receive memantine or risperidone for 8 weeks. Four scales were used to assess patients: childhood autism rating scale (CARS), ABC, CGI-I, and CGI-S. Results of ABC showed that both drugs reduced the scores of "irritability," "lethargy/social withdrawal," "inappropriate speech," and "hyperactivity" subscales, but differences between the two groups for each subscale were not found to be significant. Results obtained based on CARS indicated that both drugs reduced total score and several subscales, but differences between the two groups

were not significant for each item. Results of the CGI-I demonstrated that there are no significant differences between the two groups. Side effects of memantine included somnolence, insomnia, apnea at the beginning of speaking, nausea, deterioration of stuttering, and decrease in appetite. Memantine causes aggravation of some symptoms, which included throwing objects, impulsive behaviors, hyperactivity, agitation, and pertinacity. Side effects of risperidone were increased appetite, somnolence, fever, indifference to self-defense, enuresis, drooling, nasal congestion, and fatigue.

This study suggests that memantine may have beneficial effects in the treatment of many autism core symptoms. Nevertheless, memantine may be considered as a potential medication in the treatment of those autistic children who do not respond or cannot tolerate side effects of risperidone.

20.3.5 N-Acetylcysteine

N-acetylcysteine (2-acetamido-3-sulfanylpropanoic acid) is a derivative of the endogenous amino acid L-cysteine, which is a precursor of the antioxidant enzyme glutathione. The glutathione acts as the body's defense mechanism against the oxidant stress in several metabolic and pathological reactions. It exerts its antioxidant effects by regulating oxidative metabolism and glutamate transmission and plays a rate-limiting role in the synthesis of glutathione (Naveed et al. 2017).

N-acetylcysteine has been found to permeate the blood–brain barrier and has good bioavailability. In the brain N-acetylcysteine is oxidized from L-cysteine to cystine, facilitating its uptake by glial cells and consequently allowing the glutamate release, which in turn stimulates inhibitory glutamate receptors (Wink et al. 2016). Due to these metabotropic glutamate receptors, there is a reduction in the vesicular release of glutamate, resulting in the decrease in glutamatergic neurotransmission (Hardan et al. 2012). This mechanism of action of N-acetylcysteine is particularly important because of its effect on glutaminergic neurons in the nucleus accumbens, which is involved in the modulation of the reward and reinforcement center implicated in the addictive behaviors (Gray et al. 2012).

Wink et al. (2016) with their study focus on evaluating efficacy, safety, and tolerability of oral N-acetylcysteine, targeting core social impairment in youth with ASD. This study was a 12-week randomized, double-blind, placebo-controlled trial of oral N-acetylcysteine in 25 medically healthy children with ASD, age 4–12 years.

There was no statistically significant difference between the N-acetylcysteine and the placebo groups at week 4, week 8, or week 12 on the CGI-I primary outcome measure. At each time period, at least half of all participants were rated as having no change. On the CGI-S secondary outcome measure, no participants were noted to have increased scores suggesting clinical worsening of symptoms. There were also no differences between the N-acetylcysteine and placebo groups for those whose severity scores decreased from baseline to week 12. On the ABC, SRS, and Vineland Adaptive Behavior Scales-II – Second Edition (VABS-II) secondary outcome

measures, the employed models found no significant differences between groups in change from baseline to week 12.

To investigate the impact of N-acetylcysteine on oxidative stress markers in peripheral blood, venous blood samples were collected at screen and week 12. At week 12, the glutathione level in blood was significantly higher in the N-acetylcysteine group compared to placebo.

The results of this randomized, placebo-controlled trial indicate that N-acetylcysteine treatment was well tolerated by study participants, had the expected effect of boosting Glutathione production in peripheral blood. However, N-acetylcysteine had no significant impact on the core social impairment of ASD when compared to placebo treatment.

In another study, Nikoo et al. (2015) investigated, in a randomized, double-blind, clinical trial, the efficacy and safety of N-acetylcysteine as an add-on therapy to risperidone. The patients received N-acetylcysteine or placebo in addition to risperidone for 10 weeks.

40 children between 4 and 12 years of age completed the trial. The main tool in this study was ABC-C. Patients in the N-acetylcysteine group showed a significantly greater improvement on the Aberrant Behavior Checklist-Community subscales "irritability, hyperactivity/non-compliance." Improvements in "lethargy/social withdrawal, stereotypic behavior, and inappropriate speech" subscale scores were not significantly different between the two groups. Six adverse events (vomiting, nausea, headache, dry mouth, abdominal pain, and diarrhea) were reported, there was no statistically significant difference in the incidence of these adverse events between the two groups. All adverse events were mild and transient and did not request any kind of medical intervention.

20.3.6 D-Cycloserine

D-Cycloserine functions as a partial agonist of the glycine-binding site at NMDA receptors. By modulating glycine action as a cotransmitter at the NMDA receptors (Prosser and de Carvalho 2013), this drug is predicted to allow longer channel openings with increased Ca2+ entry, ultimately enhancing connectivity and consolidating environmentally induced plastic modifications in excitatory circuits.

The NMDA receptor is central in cortical neuroplasticity for its mechanism of long-term potentiation. The NMDA receptor is composed by 2 subunits: NR1 and NR2. D-Cycloserine acts at the glycine-binding site of the NMDA receptor, which is located at its NR1 subunit. D-Cycloserine plays a role of partial agonist of the glycine-binding site at NMDA receptors (Watson et al. 1990), it means that it acts like an agonist at low doses but has antagonistic features at high doses. This behavior is caused by its different receptor subtype selectivity and intrinsic action, which depends on various NR2 subunits (NR2A, NR2B, NR2C), the location of glutamate binding (Dravid et al. 2010). Presumably, the effects at low doses of D-Cycloserine reflect its agonistic action at the NR1/NR2C receptors, for which it has a high

affinity, while at high doses the effects might be due to antagonistic inhibition of NR1/NR2A and NR1/NR2B receptors, for which D-Cycloserine has a lower affinity (Danysz and Parsons 1998). When acts to NMDA receptors that consist of NR2C subunits, D-Cycloserine produces a 200% depolarization (compared with glycine) that is not pH-sensitive and seems not to depend on concentrations of glycine (Sheinin et al. 2001). NR2C units are mainly expressed in cerebellar structures, but are also found in the striatum, hippocampus, olfactory bulb, retrosplenial cortex, thalamus, pontine, and vestibular nuclei (Karavanova et al. 2007). Altogether, D-Cycloserine seems to have an impact on cognitive functions, mainly those associated with NMDA receptor-dependent mechanisms. Part of this effect seems to be a stabilization of NMDA receptors, with a consequent facilitation of cortical neuroplasticity (Schade and Paulus 2016).

Wink et al. (2017) and Minshawi et al. (2016) investigated, in a randomized, double-blind, placebo-controlled trial, the efficacy of low dose of D-Cycloserine given 30 min prior to weekly peer-mediated group to potentiate social skills training in youth with ASD. 67 patients completed the trial, within age range of 5–11 years. For the 10-week intervention phase of the study, participants were enrolled in a series of 17 social skill groups, each containing four children with ASD and two typically developing, age-matched peer models. Social skills intervention followed a curriculum using Applied Behavioral Analysis-based techniques designed to teach skills including greetings, understanding emotions, creative play, and social conversations. Following the 10-week intervention phase, participants received no ongoing study-related therapeutic intervention or treatment with study drug. At week 11, the SRS change scores from baseline demonstrated no statistically significant difference attributable to D-Cycloserine treatment. No significant difference noted for any of the ABC subscales between two groups.

D-Cycloserine was well tolerated, irritability was the most frequently reported adverse effect in both groups. There was no statistically significant difference in number of reported adverse events between groups.

The second trial evaluated blinded week 22 durability of treatment data collected following a 10-week randomized, double-blind, placebo-controlled D-Cycloserine plus peer-mediated social skills group intervention in 60 high functioning youth with ASD.SRS total raw scores from week 11 to week 22 demonstrate that the D-Cycloserine group decreased significantly compared to the placebo group; in particular, the social cognition subscale showed the greatest between groups difference. There were no statistically significant differences between groups on the social awareness, social communication, social motivation, and autistic mannerism subscales.

Adjunctive D-Cycloserine increased the sustained benefit from short-term social skills intervention 3 months after treatment cessation. Additionally, the safety and time limited nature of this drug treatment, as demonstrated by the limited adverse effects reported by study participants, indicates that D-Cycloserine may be a safe and effective strategy to enhance the durability of therapy impact in youth with ASD.

In another study, Urbano et al. (2015) tested D-Cycloserine in a double-blind randomized 10-week trial; the trial consisting of 8 weeks of active drug at either weekly or daily dosing and a 2-week follow-up visit.

Twenty subjects completed the study, within the age range of 15–25 years. All subjects maintained a stable medication and therapy regimen throughout the trial. Most subjects were taking a serotonin-enhancing drug such as a selective serotonin reuptake inhibitor, buspirone, or clomipramine and/or a stimulant such as extended-release methylphenidate, methylphenidate, atomoxetine, or dextroamphetamine amphetamine.

Three subjects were taking low-dose risperidone or aripiprazole, one subject was taking clonidine, and one subject was taking oxcarbazepine.

SRS was used to assess severity of social impairment in this trial. Both daily and weekly dosing strategies showed a significant downward trend when modeled separately. D-Cycloserine caused statistically and clinically significant improvement with no differentiation between dosing strategies on the Social Responsiveness Scale and the Aberrant Behavior Checklist before and after D-cycloserine administration. D-Cycloserine was very well tolerated; only transient spontaneously recorded side effects were noted by the patients and their caregivers, which were not reasons for interruption of the therapy. There was a softly higher incidence of side effects in the daily dosage group than the weekly dosage group, but the difference was not found to be statistically significant. The side effects were: mild periodic hand tremors, nervousness, tired, trouble sleeping, bad dreams, headaches, increased appetite, decreased appetite, irritable and feeling more down than usual.

The results suggest that a once-weekly pulsed dosing strategy can be adopted in future clinical trials, which will enhance compliance and minimize the potential side effects. D-Cycloserine was safe and well tolerated in this study sample (Table 20.2).

20.4 Conclusion and Future Perspective

In conclusion, all the trials on glutamatergic modulators are promising, but to date, there is insufficient evidence to clearly support the efficacy of these drugs in ASD.

These modulators appear to be safe, but their effectiveness remained unproven to treat the core symptoms of ASD.

Several results seem to be particularly promising.

Actually irritability and hyperactivity seem to decrease ASD patients treated with N-acetylcysteine associated with risperidone, and in ASD patients treated with minocycline added to risperidone.

Memantine showed similar effects to risperidone in controlling irritability and seems to be an interesting alternative drug in patients who don't tolerate risperidone.

Table 20.2 Treatment and modulators of glutamatergic system for ASD

Minocycline	Second generation tetracycline antibiotic agent; it acts bacteriostatically against a broad spectrum of germs; it seems to have beneficial effects on anti-inflammation of the central nervous system, on microglial activation, and on neuroprotection
Riluzole	It is a benzothiazole with anxiolytic, neuroprotective, anticonvulsant, and anesthetic properties. Its effects are mediated by blockade of glutamate transmission, the stabilization of sodium channels and blockage of gamma-aminobutyric acid reuptake.
Modafinil	It is a psychostimulant which works indirectly on the glutamate and GABA receptors. This drug stimulates the release of glutamate in both the hippocampus and the thalamus; additional indirect modulation of neurotransmission includes an increase in dopamine, noradrenaline, and serotonin secretion. Its precise mechanism of action remains unclear
Memantine	It antagonizes the action of glutamate and its receptors, most likely mediated principally through the voltage-dependent blockade of current flow through N-methyl-D-aspartate (NMDA) receptor channels
N-acetylcysteine	It is a derivative of the endogenous amino acid L-cysteine, which is a precursor of the antioxidant enzyme glutathione. It exerts its antioxidant effects by regulating oxidative metabolism and decreases glutamatergic neurotransmission and plays a rate-limiting role in the synthesis of glutathione
D-Cycloserine	It functions as a partial agonist of the glycine binding site at NMDA receptors. This drug is predicted to allow longer channel openings with increased Ca2+ entry, ultimately enhancing connectivity and consolidating environmentally induced plastic modifications in excitatory circuits

Treatments with D-Cycloserine showed mild positive effects in individuals with ASD mainly on social deficit and tend to show more and longer effectiveness compared to placebo.

On the contrary, despite the previous promising data, Modafinil did not confirm positive effects on social impairment in ASD.

In conclusion, since glutamatergic modulators show suggestive and still promising effects, new researches would be needed to detect more specific pathways, early in life, in order to intervening when the brain is most plastic. Current epigenetic knowledge seems to support the view that behavioral, environmental, and psychological interventions in addition to pharmacological treatments may cooperate in brain modulation and plasticity in the first years of life and may improve ASD long-term prognosis.

Notably, most clinical studies present significant limitations in terms of low homogeneity of populations, sample size, and duration of studies.

Future trials on larger and homogeneous sample are needed for a better comprehension of real and specific effectiveness of these promising drugs in the treatment of ASD.

Declaration of Interest None.

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