GLUTAMATE-RELATED BIOMARKERS FOR NEUROPSYCHIATRIC DISORDERS

EDITED BY: Hsien-Yuan Lane, Chieh-Hsin Lin and Kenji Hashimoto
PUBLISHED IN: Frontiers in Psychiatry

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-379-1
DOI 10.3389/978-2-88963-379-1

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

GLUTAMATE-RELATED BIOMARKERS FOR NEUROPSYCHIATRIC DISORDERS

Topic Editors:
Hsien-Yuan Lane, China Medical University, Taiwan
Chieh-Hsin Lin, Kaohsiung Chang Gung Memorial Hospital, Taiwan
Kenji Hashimoto, Chiba University, Japan

Citation: Lane, H.-Y., Lin, C.-H., Hashimoto, K., eds. (2020). Glutamate-Related Biomarkers for Neuropsychiatric Disorders. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88963-379-1

Table of Contents

frontiers in Psychiatry

EDITORIAL
published: 05 December 2019
doi: 10.3389/fpsyt.2019.00904

Editorial: Glutamate-Related Biomarkers for Neuropsychiatric Disorders

Chieh-Hsin Lin[1,2,3], *Kenji Hashimoto*[4] *and Hsien-Yuan Lane*[5,6,7*]

[1] Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan, [2] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, [3] School of Medicine, Chang Gung University, Taoyuan, Taiwan, [4] Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan, [5] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, [6] Department of Psychiatry & Brain Disease Research Center, China Medical University Hospital, Taichung, Taiwan, [7] Department of Psychology, College of Medical and Health Sciences, Asia University, Taichung, Taiwan

Keywords: glutamate, NMDA, schizophrenia, depression, Alzheimer's disease, autoimmune encephalitis, autism, addiction

Editorial on the Research Topic

Glutamate-Related Biomarkers for Neuropsychiatric Disorders

OPEN ACCESS

Edited by:
Ming D. Li,
Zhejiang University, China

Reviewed by:
Glen Bryan Baker,
University of Alberta, Canada

****Correspondence:***
Hsien-Yuan Lane
hylane@gmail.com

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *24 September 2019*
Accepted: *15 November 2019*
Published: *05 December 2019*

Citation:
Lin C-H, Hashimoto K and Lane H-Y (2019) Editorial: Glutamate-Related Biomarkers for Neuropsychiatric Disorders. Front. Psychiatry 10:904. doi: 10.3389/fpsyt.2019.00904

The glutamate receptor plays an important role in synaptic plasticity, learning, memory, and the pathogenesis and pathophysiology of numerous neuropsychiatric disorders such as schizophrenia, depression, addiction, autism, autoimmune encephalitis, and neurodegenerative diseases such as Alzheimer's disease.

For example, both ionotropic (especially NMDA and AMPA) and metabotropic (mGLU) receptors were reported to be involved in schizophrenia. In details, taking NMDA receptors (NMDARs) as an example, the receptors are widely distributed in most major organs (including heart, ovary, kidney, gastrointestinal system, lung, and etc.) and tissues (including ganglia cells, nerve fibers, blood vessels, enteroendocrine cells, liver, mast cells, inflammatory cells and etc.). The NMDAR has binding sites not only for glutamate or aspartate, but also a separate coagonist site for the endogenous ligands, D-serine, D-alanine, and glycine. Occupancy of the coagonist site can increase the frequency of opening of the channels activated by NMDAR agonists, facilitating excitatory neurotransmission in the brain. In fact, the binding of both glycine (or D-serine, D-alanine) and glutamate is required to open the NMDAR channel ionophore.

Some of the NMDARs isolated from peripheral tissues have been cloned and sequenced. These sequences correspond with NMDARs that have been cloned in the CNS. Further physiological and pharmacological experiments support the hypothesis that NMDARs in the periphery have similar properties to those in the CNS or expressed in host cells transfected with cloned subunits. Physiological studies with agonists and antagonists of the NR1 subunit of the NMDAR in the pig ileum have shown that these receptors are similar to those characterized in the CNS.

Direct measurement of physiological changes and NMDAR-related biomarkers in human brains is nearly impossible because of invasive testing procedures and ethical issues. Thus, developing accessible peripheral or brain image biomarkers become more important for mental illness. Peripheral gene expression may be a useful surrogate for gene expression in the CNS when the relevant gene is expressed in both. Lymphocytes or white cells have been suggested to be a neural

probe because numerous studies showed similarities between receptor expression and mechanisms of transduction processes of cells in the nervous system (e.g. neurons and glia) and lymphocytes. Blood-derived RNA has also become a convenient alternative to traditional tissue biopsy-derived RNA. Therefore, peripheral gene expressions as well as other peripheral biomarkers might have potential to be surrogate CNS biomarkers for disorders, their outcomes, and treatment responses in the CNS.

Among the 12 articles of this research topic *Glutamate-Related Biomarkers for Neuropsychiatric Disorders*, the first paper *Elevated Glutamate and Glutamine Levels in the Cerebrospinal Fluid of Patients With Probable Alzheimer's Disease and Depression*, by Madeira et al., reports increased CSF levels of glutamate and glutamine in Alzheimer's disease and major depressive disorder compared to healthy controls and to patients with normal pressure hydrocephalus. Importantly, lower and Innotest amyloid tau index (IATI) scores were correlated with higher glutamate and glutamine levels in their study cohort. Further, in subjects without dementia (Mini-Mental State Examination [MMSE] above 23), lower MMSE scores were associated with higher glutamate and glutamine levels.

Accumulating evidence suggests that dysfunctions in in the glutamate-glutamine cycle also play a role in the pathophysiology of schizophrenia. Madeira et al. demonstrated altered blood levels of glutamate and glutamine in patients with schizophrenia in their another study, *Blood Levels of Glutamate and Glutamine in Recent Onset and Chronic Schizophrenia*. They indicate that circulating glutamine/glutamate ratios rise at the onset of illness but fall with progression of the disorder.

In a brief review, *Astrocytic Regulation of Glutamate Transmission in Schizophrenia*, Mei et al. updated findings on the crucial role of glutamate uptake and astrocyte-derived D-serine in the pathophysiology of schizophrenia. Neuroimaging and neurochemical biomarkers based on glutamate transmission and metabolism have also shown translational potential. Further elucidation of neuron-astrocyte interaction during the aforementioned processes will facilitate the development of novel diagnostic and therapeutic approaches for schizophrenia and related disorders.

Not only ionotropic but also metabotropic glutamate (mGlu) receptors are regarded as novel drug targets for the treatment of schizophrenia. Nicoletti et al. reviewed the development of selective ligands of individual mGlu receptors as new therapy for schizophrenia in the article *Targeting mGlu Receptors for Optimization of Antipsychotic Activity and Disease-Modifying Effect in Schizophrenia*. Subtype-selective mGlu receptor ligands thereby offer the potential of a precision medicine based pharmacological approach for various domains of schizophrenia. Moreover, some mGlu receptor ligands may exert a positive influence on neuroinflammation by modulating microglial function or other mechanisms.

While glutamate is the agonist of the NMDAR, D-serine appears to be the most potent coagonist. The review article (*D-Serine: Potential Therapeutic Agent and/or Biomarker in Schizophrenia and Depression?*) by Mackay et al. indicates that neurons are more relevant to D-serine action than originally thought. Interestingly, D-serine appears to have antipsychotic and antidepressive properties. D-serine may be also a biomarker for antidepressant response to ketamine. Preclinical and clinical studies reveal that D-serine may be a potential cognitive enhancer, deserving further study.

Besides D-serine and D-alanine, another endogenous D-amino acid in the mammalian brain, D-aspartate, can influence NMDAR-mediated transmission. Errico et al. in their review, *The Emerging Role of Altered D-Aspartate Metabolism in Schizophrenia: New Insights From Preclinical Models and Human Studies*, indicated that increased levels of the NMDAR agonist, D-aspartate, impact on a series of functional and structural phenotypes relevant to schizophrenia. Moreover, these findings suggest a possible role of dysregulated embryonic D-aspartate metabolism in schizophrenia pathogenesis, and consequently propose the potential of D-aspartate as a novel therapy or prevention for this neurodevelopmental brain disorder.

D-amino acid oxidase activator (DAOA, also known as G72) protein regulates D-serine neurotransmission. Previous study found that the peripheral G72 protein expression is distinctively higher in patients with schizophrenia than in healthy individuals. Lin and Lin in their study *Combination of G72 Genetic Variation and G72 Protein Level to Detect Schizophrenia: Machine Learning Approaches*, demonstrated that G72 protein alone, even without adding G72 SNPs, can discriminate schizophrenia patients from healthy subjects, with adequate power. They also suggest a combination of logistic regression and naive Bayes models to establish an optimal model to predict schizophrenia.

Dysfunctional glutamatergic neurotransmission is implicated in the etiology of not only schizophrenia but also major depressive disorder. In the review article *Glutamatergic Dysfunction and Glutamatergic Compounds for Major Psychiatric Disorders: Evidence From Clinical Neuroimaging Studies*, Li et al. indicated that ketamine, an NMDAR antagonist, has displayed its rapid antidepressant efficacy for treatment-resistant depression. Many compounds which modulate the glutamatergic transmission have also shown potential in the treatment of major psychiatric disorders, via approaches such as magnetic resonance spectroscopy, positron emission tomography/single-photon emission computed tomography, and paired-pulse transcranial magnetic stimulation.

On the other hand, ketamine is also a common drug of abuse, while disrupted oxytocin system is involved in the development of addiction. In the original study *Decreased Blood Levels of Oxytocin in Ketamine-Dependent Patients During Early Abstinence*, Huang et al. found that the oxytocin level was significantly lower in ketamine-dependent patients and the level did not normalize after early abstinence. Lower oxytocin might be also related with anxious phenotype of ketamine dependence. These results suggest the potential of therapeutic use of oxytocin for treating ketamine dependence.

Previous studies have demonstrated that plasma asymmetric dimethylarginine (ADMA) was increased in patients with schizophrenia and correlated with cognitive dysfunction. In the study *Treatment Responses of Cognitive Function and Plasma*

Asymmetric Dimethylarginine to Atypical Antipsychotic in Patients With Schizophrenia, Yu et al. found that atypical antipsychotic treatment in acutely exacerbated schizophrenia patients improved psychiatric symptoms and cognitive function (especially working memory and attention), in parallel with decreased plasma ADMA levels, implying that plasma ADMA may be a potential indicator of cognitive recovery in schizophrenia.

In the review, *Autism Associated With Anti-NMDAR Encephalitis: Glutamate-Related Therapy*, Tzang et al. addressed the role of autoimmune dysfunction in autism. The anti-NMDAR autoantibody can lead to dysfunctional glutamate neurotransmission in the brain that manifests as various psychiatric symptoms such as psychosis and personality changes. Glutamate-related therapy primarily normalizes glutamate neurotransmission and can be a new adjuvant intervention alongside antipsychotics for treating autoimmune autism.

Acupuncture has been regarded as a complementary therapy for neuropsychiatry disorders. In the article, *The Effects of Acupuncture on Glutamatergic Neurotransmission in Depression, Anxiety, Schizophrenia, and Alzheimer's Disease: A Review of the Literature Review*, Tu et al. reported the current evidence for the treatment efficacy of acupuncture in depression, anxiety, schizophrenia, and Alzheimer's disease. Further, they discuss the ways in which acupuncture treatment can potentially modulate glutamate receptors and excitatory amino acid transporters.

Overall, to promote the development of glutamate-related biomarkers for neuropsychiatric disorders, this research topic gathers a comprehensive body of review and original articles to update the current state of this increasingly important theme. Further basic, preclinical and clinical studies are needed to elucidate the possible mechanisms of glutamate-related dysfunction in neuropsychiatric disorders and to establish clinical applications for diagnoses and treatments of these disorders.

AUTHOR CONTRIBUTIONS

C-HL and H-YL wrote the first draft of the manuscript, and KH provided opinions on it. All authors read and approved the submitted version.

FUNDING

This work was partly supported by Ministry of Science and Technology, Taiwan (MOST 107-2632-B-039-001; 108-2314-B-039-002; 108-2622-B-039-001-CC2; 108-2628-B-182A-002), National Health Research Institutes (NHRI-EX108-10731NI; NHRI-EX108-10816NC), China Medical University Hospital, Taiwan (DMR-105-076), Chang Gung Memorial Hospital, Taiwan (CMRPG8G1391), and Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW108-TDU-B-212-133004).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 06 November 2018
doi: 10.3389/fpsyt.2018.00561

Elevated Glutamate and Glutamine Levels in the Cerebrospinal Fluid of Patients With Probable Alzheimer's Disease and Depression

Caroline Madeira[1], *Charles Vargas-Lopes*[1], *Carlos Otávio Brandão*[2], *Taylor Reis*[2], *Jerson Laks*[2], *Rogerio Panizzutti*[1,2]* *and Sergio T. Ferreira*[3,4]*

[1] *Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil,* [2] *Institute of Psychiatry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil,* [3] *Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil,* [4] *Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

OPEN ACCESS

Edited by:
Hsien-Yuan Lane,
China Medical University, Taiwan

Reviewed by:
Kenji Hashimoto,
Chiba University, Japan
Chih-Chiang Chiu,
Taipei City Psychiatric Center, Taiwan
Cheng-Sheng Chen,
Kaohsiung Medical University Hospital, Taiwan

***Correspondence:**
Rogerio Panizzutti
rogerio@icb.ufrj.br
Sergio T. Ferreira
ferreira@bioqmed.ufrj.br

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *19 June 2018*
Accepted: *17 October 2018*
Published: *06 November 2018*

Citation:
Madeira C, Vargas-Lopes C, Brandão CO, Reis T, Laks J, Panizzutti R and Ferreira ST (2018) Elevated Glutamate and Glutamine Levels in the Cerebrospinal Fluid of Patients With Probable Alzheimer's Disease and Depression. Front. Psychiatry 9:561. doi: 10.3389/fpsyt.2018.00561

Recent evidence suggests that Alzheimer's disease (AD) and depression share common mechanisms of pathogenesis. In particular, deregulation of glutamate-mediated excitatory signaling may play a role in brain dysfunction in both AD and depression. We have investigated levels of glutamate and its precursor glutamine in the cerebrospinal fluid (CSF) of patients with a diagnosis of probable AD or major depression compared to healthy controls and patients with hydrocephalus. Patients with probable AD or major depression showed significantly increased CSF levels of glutamate and glutamine compared to healthy controls or hydrocephalus patients. Furthermore, CSF glutamate and glutamine levels were inversely correlated to the amyloid tau index, a biomarker for AD. Results suggest that glutamate and glutamine should be further explored as potential CSF biomarkers for AD and depression.

Keywords: Alzheimer's disease, depression, glutamate, glutamine, cerebrospinal fluid, innotest amyloid tau index

INTRODUCTION

Epidemiological and clinical studies suggest an association between Alzheimer's disease (AD) and depression (1, 2), the latter being a risk factor for development of AD and other forms of dementia (3–5). About half of the patients with major depression show cognitive impairment that can be persistent and last even after remission of the acute phase of symptoms (6). This could mean cognitive impairment precedes or predisposes to depression or, alternatively, that depression produces persistent cognitive deficits (6, 7). Recent studies have further suggested that similar pathogenic mechanisms may underlie cognitive changes in depression and in AD. Indeed, we have demonstrated that soluble oligomers of the amyloid-β peptide (AβOs), neurotoxins that accumulate in the AD brain and are thought to cause synapse failure and memory loss in AD (8, 9), induce depressive-like behavior in mice (10, 11), providing insight into molecular/cellular mechanisms potentially connecting both disorders.

Glutamate is the major excitatory neurotransmitter in the mammalian brain. However, glutamate at high concentrations in the synaptic cleft is toxic and may result in neuronal death, a phenomenon generally termed excitotoxicity (12). Excitotoxicity mediated by aberrant activation of glutamate receptors, notably of N-methyl-D-aspartate (NMDA) receptors, has been related to the neuropathology of AD (13–16). Consistent with a role of abnormal NMDA receptor function in

AD, memantine, an NMDA receptor blocker, is one of the few drugs in clinical use for treatment of moderate to severe AD (17, 18). Interestingly, NMDA receptor antagonists, in particular ketamine, also produce rapid and consistent antidepressant action in subjects with major depression (19, 20).

To prevent excitotoxicity after physiological neurotransmission, glutamate is rapidly removed from the synaptic cleft and converted into glutamine by glutamine synthetase in glial cells (21). Glutamine is then transported back to the presynaptic neuron, where it is converted to glutamate by glutaminase (22). Dysfunction in the glutamate-glutamine cycle could contribute to excitotoxicity mediated by glutamate (22).

Changes in glutamate and glutamine may occur in AD and depression, previous studies of glutamate and glutamine levels in the cerebrospinal fluid (CSF) present controversial findings. Some studies have reported that CSF glutamate levels in AD patients were higher than in the controls (23, 24) or MCI (25), whereas others studies have found decreased (26, 27) or unchanged glutamate levels in AD patients compared to healthy controls (28, 29). Studies that measured CSF glutamine levels have also reported controversial results, with studies reporting increased (30), decreased (28, 31) or no change in glutamine levels in AD patients compared with controls (23, 24, 26).

Controversial findings have also been observed in CSF glutamate and glutamine levels of patients with depression. One study found an increase in CSF glutamine levels (32), whereas another study showed a decrease in CSF glutamate levels in patients with depression compared to controls (33). Moreover, recent studies reported no differences in CSF glutamate and glutamine levels in patients with depression compared to controls (34, 35).

Here, we investigated whether changes in brain levels of glutamate and glutamine are present in major depression and AD. We have studied glutamate and glutamine levels in the CSF of age-matched patients with probable AD or major depression compared to two control groups: healthy subjects and patients with an unrelated neurological condition (normal pressure hydrocephalus). We further investigated the correlation between glutamate and glutamine levels, Mini-Mental State Examination (MMSE) scores, and the Innotest amyloid tau index, a biomarker for AD (36, 37).

MATERIALS AND METHODS

Human Subjects

The current study was approved by the Committee of Research Ethics involving human subjects of the Institute of Psychiatry of Federal University of Rio de Janeiro (protocol # 32liv2/07). Subjects provided written informed consent before study admission.

All subjects underwent an evaluation comprising full medical history, physical and neurological examination, laboratory tests and neuropsychological assessments. The complete work up is detailed elsewhere (38). The severity of dementia was classified according to the Clinical Dementia Rating (CDR) (39). Mini-Mental State Examination (MMSE) (40) was additionally used to evaluate cognitive state. Exclusion criteria included more than 10 packs/year of cigarette smoking, alcohol abuse or other current or previous psychiatric or clinical disorder. The probable AD group included 21 subjects recruited from the AD Center of the Institute of Psychiatry of the Federal University of Rio de Janeiro. Subjects were diagnosed according to National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), Alzheimer's Disease and Related Disorders Association (ADRDA) and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (41). The depression group included 9 subjects with major depression, recruited from the Institute of Psychiatry of the Federal University of Rio de Janeiro and diagnosed according to DSM-IV criteria. The severity of depression was measured by the Brazilian version of the Hamilton Depression Scale (HAM-D) (42–44). Only patients in the first episode of major depression were included in the study. The healthy control group included 10 subjects without any clinical disease or neuropsychiatric disorder. We also studied an additional control group including 9 patients with hydrocephalus, diagnosed according to International Classification of Diseases (ICD-10) (45). Both healthy and hydrocephalus subjects were recruited at Neurolife Laboratory, a private clinic specialized in CSF analysis in the city of Rio de Janeiro. Selected characteristics of each studied group are presented in **Table 1**. Detailed demographics of individual subjects are presented in **Table S1**.

Psychotropic medications used by probable AD patients were: rivastigmine (47.6%; $n = 10$), risperidone (38.1%; $n = 8$), memantine (28.6%; $n = 6$), donepezil (23.8%; $n = 5$), clonazepam (19.0%; $n = 4$), citalopram (4.8%; $n = 1$), trazodone (4.8%; $n = 1$), biperiden (4.8%; $n = 1$), escitalopram (4.8%; $n = 1$), and mirtazapine (4.8%; $n = 1$). Two (9.5%) patients with probable AD were not taking any medication at the time of the study. Medications used by major depression patients were: citalopram (11.1%; $n = 1$), clonazepam (33.3%; $n = 3$), fluoxetine (22.2%; $n = 2$), desvenlafaxine (11.1%; $n = 1$), paroxetine (11.1%; $n = 1$), buspirone (11.1%; $n = 1$), sertraline (11.1%; $n = 1$), venlafaxine (11.1%; $n = 1$). One (11.1%) patient with major depression was not taking any medication at the time of the study.

CSF Collection

CSF samples were collected by lumbar puncture in the L3-4 or L4-5 interspace at Neurolife Laboratories (Rio de Janeiro, Brazil) and immediately stored at −80°C. All lumbar punctures were performed between 10 am and noon to limit potential circadian fluctuation in CSF content.

Abbreviations: AD, Alzheimer's disease; ADRDA, Alzheimer's Disease and Related Disorders Association; ANOVA, Analysis of variance; Aβ, amyloid-β peptide; AβO, amyloid-β oligomer; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; GLX, glutamate plus glutamine concentrations; HPLC, high performance liquid chromatography; IATI, Innotest amyloid tau index; ICD-10, International Classification of Diseases; MMSE, Mini-Mental State Examination; MRS, magnetic resonance spectroscopy; NINCDS, National Institute of Neurological and Communicative Disorders and Stroke; NMDA, N-methyl-D-aspartate; P-tau181, phosphorylated tau; T-tau, total tau.

TABLE 1 | Characteristics of study subjects.

	Control	AD	Major depression	Hydrocephalus	Statistics
Age, years	70.7 (6.3)	72.1 (8.4)	69.8 (5.8)	74.6 (7.4)	0.71 (0.55)
Sex, male/female	3/7	9/12	0/9	5/4	5.90 (0.01)*
Education, years	7.9 (5.1)	4.8 (4.8)	2.7 (2.6)	7.6 (5.7)	2.66 (0.06)
Disease duration, months	N.A.	44.8 (28.2)	N.A.	24.7 (13.6)	N.A.
MMSE	27.1 (1.3)	12.7 (6.2)[a]	24.4 (2.2)	27.2 (1.8)	39.66 (0.0001)*
IATI	1.95 (0.40)	0.74 (0.34)[b]	1.58 (0.62)	1.67 (0.56)	18.29 (0.0001)*
HAM-D	N.A.	N.A.	15.2 (2.0)	N.A.	N.A.
Glutamate, μmol/l	6.16 (3.19)	17.28 (1.99)[c]	16.31 (3.81)[d]	9.05 (2.06)	50.83 (0.0001)*
Glutamine, μmol/l	359.3 (102.1)	534.0 (146.8)[e]	493.7 (151.8)	359.6 (108.2)	5.93 (0.002)*
Glutamate/glutamine ratio	0.0158 (0.0060)	0.0334 (0.0079)[f]	0.0325 (0.0082)[g]	0.0260 (0.0043)[h]	15.34 (0.0001)*

Values are presented as means (standard deviation). Statistical significance of differences between groups was assessed by one-way ANOVA, F (p-value), followed by Bonferroni adjustment for multiple comparisons, except for sex distribution, which was assessed by the Chi-Square Test, X^2 (p-value). Asterisks indicate statistically significant differences.
AD, Alzheimer's disease; MMSE, Mini Mental State Examination; IATI, Innotest amyloid tau index; HAM-D, Hamilton Depression Scale; N.A., Not applicable or not available.
[a] AD significantly different from control, hydrocephalus and depression (P = 0.0001).
[b] AD significantly different from control, hydrocephalus and depression (P = 0.0001).
[c] AD significantly different from control and hydrocephalus (P = 0.0001).
[d] Depression significantly different from control and hydrocephalus (P = 0.0001).
[e] AD significantly different from control and hydrocephalus (P = 0.008 and P = 0.012, respectively).
[f] AD significantly different from control (P = 0.0001).
[g] Depression significantly different from control (P = 0.0001).
[h] Hydrocephalus significantly different from control (P = 0.018).

Glutamate and Glutamine Measurements in CSF

Glutamate and glutamine levels in CSF were measured by high performance liquid chromatography (HPLC) as previously described (46–48).

Determination of the Amyloid Tau Index

CSF concentrations of Aβ1-42, total tau (T-tau) and phosphorylated tau (P-tau181) were measured using commercially available enzyme-linked immunosorbent assays (ELISA INNOTEST p-tau-181, INNOTEST htau, INNOTEST β-amyloid (1-42) kits; Innogenetics, Gent, Belgium) according to manufacturer's instructions. The Innotest amyloid tau index (IATI) is a score that combines CSF levels of Aβ1-42 and T-tau (36, 37) and was calculated as:

$$\text{IATI} = \text{A}\beta_{1-42}/(240+1.18*\text{T-tau}).$$

Statistical Analysis

Results are presented as means (±S.D.) unless otherwise indicated. Statistical significances between groups were determined by one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons. Sex distribution was studied using Chi-Square. Interactions between years of education, age, disease duration and levels of glutamate or glutamine were studied using correlation analysis and were demonstrated using Pearson's correlation coefficient (r).

RESULTS

Patient groups did not differ in age, but the sex distribution was significantly different between groups (**Table 1**). However, logistic regression analysis did not reveal significant interactions between sex and levels of glutamate or glutamine ($\chi^2 = 0.21$, $p = 0.64$ for glutamate; $\chi^2 = 0.93$, $p = 0.33$ for glutamine). Groups also differed in terms of numbers of years of education (**Table 1**). However, no significant correlations were found between years of education and levels of glutamate or glutamine ($r = -0.19$, $p = 0.18$ for glutamate; $r = -0.02$, $p = 0.87$ for glutamine). Moreover, glutamate and glutamine levels were not significantly correlated to age ($r = 0.04$, $p = 0.78$ for glutamate; $r = 0.22$, $p = 0.12$ for glutamine) or disease duration ($r = 0.22$, $p = 0.23$ for glutamate; $r = 0.03$, $p = 0.86$ for glutamine). None of the medications in use by the patients showed a significant effect on glutamate and glutamine levels (**Table S2**).

Mean CSF glutamate levels were significantly higher in patients with probable AD compared to healthy controls and hydrocephalus patients ($F = 50.8$, $p < 0.0001$) (**Table 1**; **Figure 1A**). Interestingly, CSF glutamate levels in the major depression group were similar to the levels found in the probable AD group (**Table 1**, **Figure 1A**). The sensitivity and specificity were 95.2 and 100%, respectively (using a cutoff of 13.63 μmol glutamate/l) for the diagnosis of probable AD compared to healthy controls (AUC = 0.99, $p < 0.0001$). The corresponding ROC curve is presented in **Figure S1**.

Mean glutamine levels were significantly higher in patients with probable AD than in healthy controls and in the hydrocephalus group ($F = 5.92$, $p = 0.002$). Moreover, the mean CSF glutamine level in the major depression group was similar to the mean level found in the group of patients with probable AD (**Table 1**; **Figure 1B**).

The glutamate/glutamine ratio, an index of glutamine-glutamate cycle in the brain (49), was significantly higher in all three patient groups (probable AD, major depression

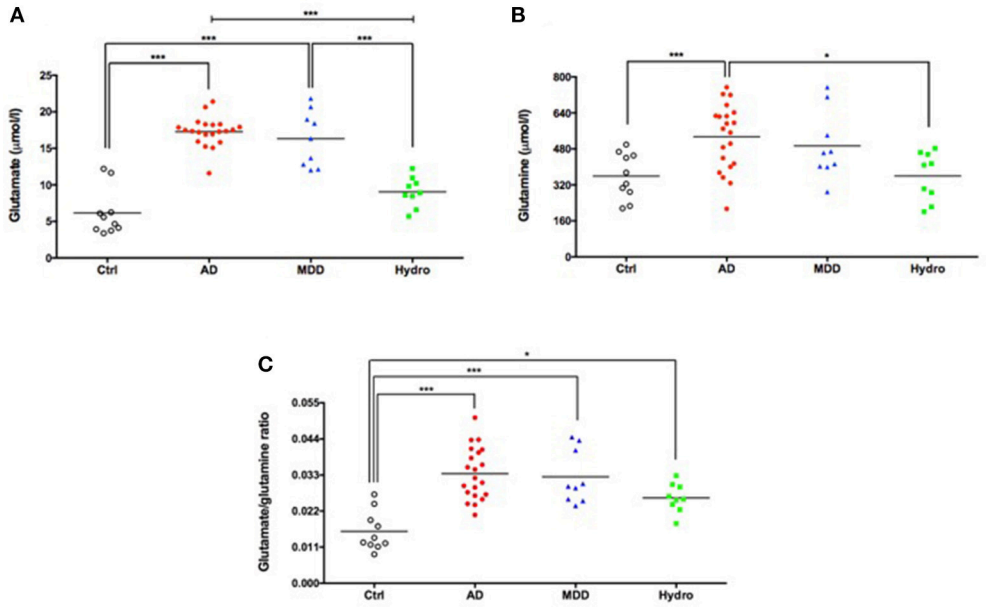

FIGURE 1 | Increased CSF levels of glutamate **(A)**, glutamine **(B)** and glutamate/glutamine ratio **(C)** in patients with probable AD and major depression. Symbols correspond to individual subjects. Horizontal lines represent mean values for each group. Statistical significances assessed by one-way ANOVA followed by Bonferroni adjustment for multiple comparisons. $^{*}P < 0.05$; $^{***}P < 0.001$. AD, probable Alzheimer's disease; Ctrl, healthy controls; MDD, major depressive disorder; Hydro, hydrocephalus.

and hydrocephalus) compared to healthy controls (**Table 1**; **Figure 1C**). Thus, while measurements of CSF glutamate alone robustly separated probable AD and depressive patients from controls and hydrocephalus patients, the glutamate/glutamine ratio was elevated in all three disorders compared to controls, suggesting it was a less specific biomarker for AD and depression.

As expected, the mean MMSE score was significantly lower in patients with probable AD than in healthy controls, major depression and hydrocephalus (**Table 1**). Interestingly, in subjects without dementia (MMSE above 23) (50), lower MMSE scores were significantly associated with higher CSF glutamate ($r = -0.51$, $p = 0.006$) (**Figure 2A**) and glutamine levels ($r = -0.47$, $p = 0.013$) (**Figure 2B**).

As also expected, the mean CSF Innotest amyloid tau index (IATI) was significantly lower in the AD group compared to the other three groups (**Table 1**). Remarkably, CSF glutamate levels were significantly and inversely correlated to the IATI across the four subject groups ($r = -0.45$, $p = 0.002$) (**Figure 2C**). Glutamine levels were also inversely correlated to the IATI, although the correlation was less robust and significant ($r = -0.31$, $p = 0.04$) (**Figure 2D**).

Finally, CSF glutamate and glutamine levels were analyzed in subject groups separated by their clinical dementia rating (CDR) scores. Individuals with CDR 0.5, 1, 2, and 3 showed significantly elevated mean glutamate levels compared to non-cognitively impaired individuals (CDR 0) (**Figure 3A**). On the other hand, only the group with CDR 2 exhibited significantly higher glutamine than the CDR 0 group (**Figure 3B**).

DISCUSSION

We report increased CSF levels of glutamate and glutamine in AD and major depression compared to healthy controls and to patients with normal pressure hydrocephalus. Significantly, lower MMSE and IATI scores were correlated with higher glutamate and glutamine levels in our study cohort.

The current findings are consistent with previous studies reporting increased glutamate in the CSF of patients with probable AD (23, 24). In addition, CSF glutamate levels were found to be significantly elevated in patients with AD in comparison to patients with mild cognitive impairment (25). However, other groups have reported decreased (26, 27) or unchanged glutamate levels in patients with probable AD compared to healthy controls (28, 29). Moreover, our finding of increased CSF glutamine in AD patients is in accord with a previous study (30) but differs from other studies that reported a decrease (28, 31) or no change in glutamine levels in patients with probable AD compared to healthy controls (23, 24, 26). It is noteworthy that the criteria for diagnosis of probable AD in early studies included only clinical features (51), which could lead to greater heterogeneity amongst individuals included in the studies and, hence, to greater variability in results from CSF analysis. More recent revisions in diagnostic and research criteria have advocated the use of biomarkers as closely as possible to the pathological criteria for AD so as to increase the level of certainty in the diagnosis for research purposes (52, 53). All subjects with probable AD studied here were positive for the biomarker amyloid tau index (IATI), thus showing clear evidence of both

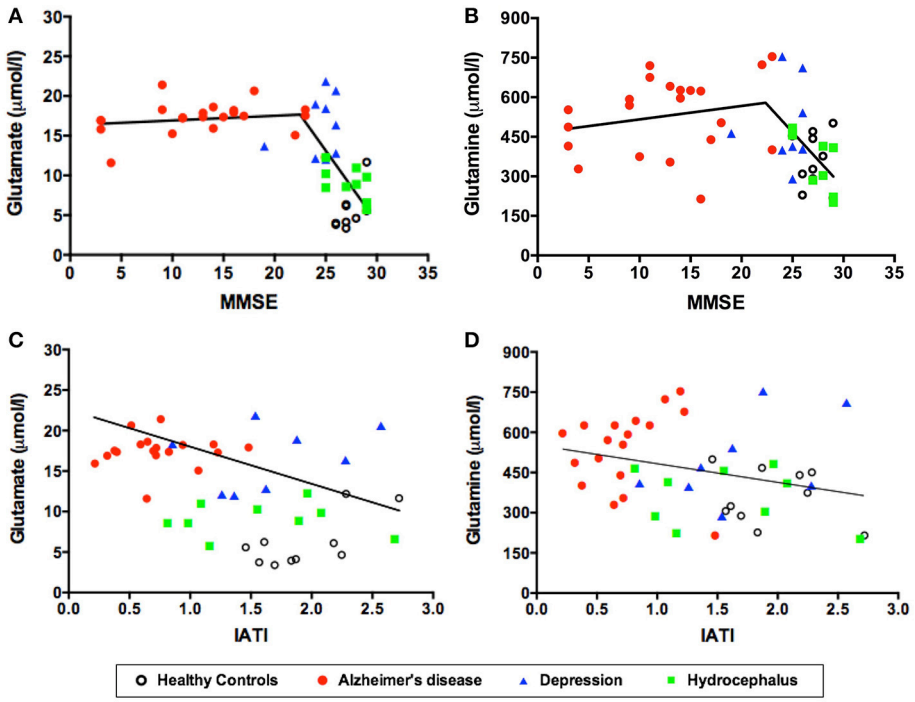

FIGURE 2 | CSF levels of glutamate **(A)** and glutamine **(B)** as a function of MMSE scores. CSF levels of glutamate **(C)** and glutamine **(D)** as a function of the IATI index. Symbols correspond to individual subjects. Statistical significances assessed by Pearson correlation. MMSE, Mini Mental State Examination; IATI, INNOTEST amyloid/tau index.

amyloid-β and tau neuropathology. Interestingly, glutamate and glutamine levels were inversely correlated with individual IATI values across all groups of subjects. We further note that we have previously reported measurements of CSF levels of D-serine, L-serine and glycine in the same patient cohort investigated in the current study (48). We found that D-serine levels in patients with probable AD were significantly higher than in healthy controls, but there were no differences in L-serine and glycine levels.

We further found elevated CSF glutamate and glutamine in major depression patients, similar to levels found in the AD group and significantly higher than the levels found in both healthy control individuals and hydrocephalus patients. Previous studies have measured glutamate and glutamine in the CSF of patients with major depression, and they report controversial findings. Levine et al. (32) reported increased glutamine levels in the CSF of depressed patients compared to controls, whereas Frye et al. (33) showed decreased glutamate levels in depression. More recent studies reported no differences in glutamate or glutamine levels in the CSF of depressed patients compared to controls (34, 35). Of note, Levine et al. (32), Frye et al. (33) and Garakani et al. (34) evaluated middle-aged adult patients, while here we studied older patients. On the other hand, Hashimoto et al. (35) also studied older patients, but the MMSE scores average of their study was slightly higher than in our study. More studies are thus warranted to clarify whether glutamate and glutamine are altered in individuals affected by depression and belonging to different age groups. Increased CSF glutamate may be related to the neurodegenerative process thought to occur in major depression. Evidence indicates that inflammation leading to neurodegeneration plays an important role in depression (54–56). Glutamate excitotoxicity may be involved in this cascade as inflammatory mediators increase glutamate release and decrease glutamate uptake in the central nervous system (57–59).

CSF glutamate and glutamine levels were similar in age-matched patients with probable AD or depression, suggesting another shared mechanism of pathogenesis in these two disorders. Accordingly, a prospective study in a large cohort suggested that depression in the older adult is a prodrome rather than a risk factor for AD (60). Given the central role of glutamatergic neurotransmission in synaptic plasticity, learning and memory (61), as well as in regulation of mood (62), the impact of altered glutamate and glutamine levels on neuropathological mechanisms connecting dementia and depression warrants further investigation. Notably, evidence from animals models indicates that AD and depression involves shared mechanisms, such as hyperactivation of NMDA receptors through increased D-serine (48), inflammatory process (10, 11) and serotonergic signaling (11).

What is the significance of increased glutamate and glutamine levels in the CSF of AD patients? The increase in CSF glutamine may be due to increased activity of aspartate aminotransferase, an enzyme that forms glutamine, in the AD brain (30). A possible mechanism to explain the increase in CSF glutamate involves the build-up of Aβ oligomers (AβOs) in the AD brain. AβOs

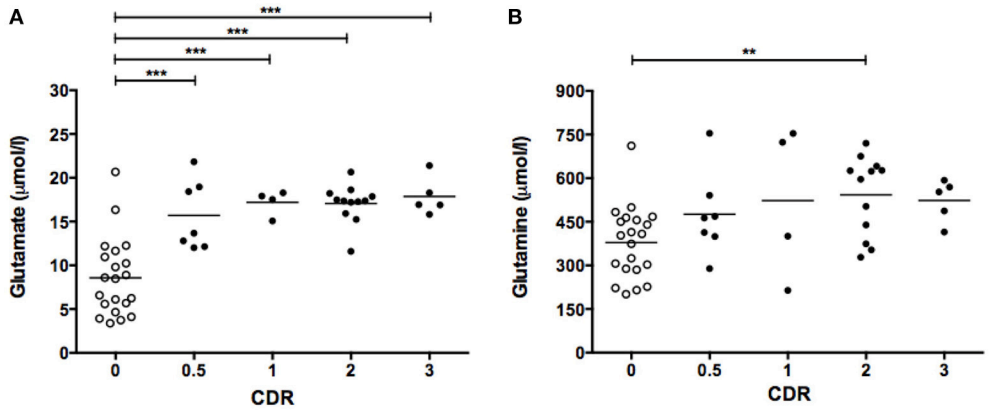

FIGURE 3 | CSF levels of glutamate **(A)** and glutamine **(B)** as a function of CDR score. Symbols correspond to individual subjects. Horizontal lines represent mean values for each group. Statistical significances assessed by one-way ANOVA followed by Bonferroni adjustment for selected groups: CDR 0.5, 1, 2, and 3 vs. CDR 0. $^{**}P < 0.01$; $^{***}P < 0.001$. CDR, Clinical Dementia Ratio.

are increasingly recognized as proximal neurotoxins in AD (8, 9, 63, 64) and oligomer levels increase in AD brains. Of note, we have previously demonstrated that AβOs cause an increase in extracellular levels of glutamate in hippocampal neurons (65), and intracerebroventricular injection of AβOs induces both depressive-like behavior and cognitive deficits in mice (10, 11, 66, 67). It may thus be that AβO-instigated increases in brain glutamate levels underlie, at least in part, cognitive and mood alterations in AD.

Interestingly, we found that glutamate and (albeit somewhat less strongly) glutamine levels are significantly and inversely associated with the MMSE score in subjects without dementia (with MMSE above 23). This implies that alterations in CSF glutamate levels sensitively correlate with sub-clinical deterioration in cognitive performance. Thus, CSF measurements of glutamate/glutamine may serve as a biomarker of subtle cognitive changes that, while still within the range of normality, may reveal underlying mechanisms of pathogenesis potentially leading to future dementia. This could be of major clinical utility in terms of detecting pre-clinical dementia, with clear implications for inclusion of subjects in clinical trials and initiation of preventive/treatment strategies prior to overt cognitive deterioration.

A limitation of the present study was the absence of males in the group of patients with major depression. We note, however, that no sex differences in glutamate and glutamine levels were found in the other three patient groups studied. Additionally, the groups of patients with probable AD and major depression showed a clear trend (albeit not statistically significant) toward lower education than the control and hydrocephalus groups. However, analysis of differences in glutamate levels using education as a covariate confirmed that differences between patient groups remained significant (data not shown). Moreover, the cohort we have studied was of modest size, and it may not be representative of the population as a whole. However, the highly significant differences we have found between groups strongly suggest that the differences may hold in larger patient groups. Nevertheless, despite the current statistically robust results, we acknowledge that this is an initial investigation and, thus, a larger study is warranted to confirm and extend the validity of our findings.

In conclusion, glutamate and glutamine levels are increased in the CSF of patients with probable AD. Significantly increased glutamate levels were detected in CDR 0.5 patients, raising the possibility that determination of CSF glutamate levels might constitute an additional biomarker for pre-clinical cognitive deterioration or early stages of dementia. Finally, our finding that glutamate and glutamine levels were also increased in the CSF of older patients with major depression suggests that these amino acids may be involved in shared mechanisms of pathogenesis between AD and major depression.

AUTHOR CONTRIBUTIONS

All authors certify that they have participated sufficiently in the work to take public responsibility for the content. RP and SF participated in the conception and design of study. CM, RP, and SF wrote the manuscript. CM and CV-L performed the analysis and interpretation of the data. CB, TR, and JL conduced analysis of patients.

ACKNOWLEDGMENTS

We thank A. C. Rangel and A. Fantinatti for administrative and technical support. We also thank Dr. L. Scoriels for review of the manuscript. This research was supported by grants from DECIT/SCTIE/MS, SESDC, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) to RP and SF; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to RP, SF, and JL; Instituto Nacional de Ciência e Tecnologia de Biologia Estrutural e Bioimagem

(INBEB) to RP; and National Institute for Translational Neuroscience to SF. RP was recipient of a Long-Term Fellowship from the Human Frontier Science Program. CM and CV-L were supported by pre-doctoral fellowships from FAPERJ and CNPq.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00561/full#supplementary-material

REFERENCES

1. Zubenko GS, Zubenko WN, McPherson S, Spoor E, Marin DB, Farlow MR, et al. A collaborative study of the emergence and clinical features of the major depressive syndrome of Alzheimer's disease. *Am J Psychiatry* (2003) 160:857–66. doi: 10.1176/appi.ajp.160.5.857
2. Gualtieri CT, Johnson LG. Age-related cognitive decline in patients with mood disorders. *Prog Neuropsychopharmacol Biol Psychiatry* (2008) 32:962–7. doi: 10.1016/j.pnpbp.2007.12.030
3. Green RC, Cupples LA, Kurz A, Auerbach S, Go R, Sadovnick D, et al. Depression as a risk factor for Alzheimer disease: the MIRAGE Study. *Arch Neurol*. (2003) 60:753–759. doi: 10.1001/archneur.60.5.753
4. Saczynski JS, Beiser A, Seshadri S, Auerbach S, Wolf PA, Au R. Depressive symptoms and risk of dementia: the Framingham Heart Study. *Neurology* (2010) 75:35–41. doi: 10.1212/WNL.0b013e3181e62138
5. Diniz BS, Butters MA, Albert SM, Dew MA, Reynolds CF 3rd. Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies. *Br J Psychiatry* (2013) 202:329–35. doi: 10.1192/bjp.bp.112.118307
6. Reppermund S, Ising M, Lucae S, Zihl J. Cognitive impairment in unipolar depression is persistent and non-specific: further evidence for the final common pathway disorder hypothesis. *Psychol Med*. (2009) 39:603–14. doi: 10.1017/S003329170800411X
7. Reppermund S, Zihl J, Lucae S, Horstmann S, Kloiber S, Holsboer F, et al. Persistent cognitive impairment in depression: the role of psychopathology and altered hypothalamic-pituitary-adrenocortical (HPA) system regulation. *Biol Psychiatry* (2007) 62:400–6. doi: 10.1016/j.biopsych.2006.09.027
8. Ferreira ST, Klein WL. The Abeta oligomer hypothesis for synapse failure and memory loss in Alzheimer's disease. *Neurobiol Learn Mem*. (2011) 96:529–543. doi: 10.1016/j.nlm.2011.08.003
9. Mucke L, Selkoe DJ. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med*. (2012) 2:a006338. doi: 10.1101/cshperspect.a006338
10. Ledo JH, Azevedo EP, Clarke JR, Ribeiro FC, Figueiredo CP, Foguel D, et al. Amyloid-beta oligomers link depressive-like behavior and cognitive deficits in mice. *Mol Psychiatry* (2013) 18:1053–4. doi: 10.1038/mp.2012.168
11. Ledo JH, Azevedo EP, Beckman D, Ribeiro FC, Santos LE, Razolli DS, et al. Cross talk between brain innate immunity and serotonin signaling underlies depressive-like behavior induced by Alzheimer's amyloid-β oligomers in mice. *J Neurosci*. (2016) 36:12106–16 doi: 10.1523/JNEUROSCI.1269-16.2016
12. Greene JG, Greenamyre JT. Bioenergetics and glutamate excitotoxicity. *Prog Neurobiol*. (1996) 48:613–34.
13. Hynd MR, Scott HL, Dodd PR. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochem Int*. (2004) 45:583–95. doi: 10.1016/j.neuint.2004.03.007
14. Dong XX, Wang Y, Qin ZH. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacol Sin*. (2009) 30:379–87. doi: 10.1038/aps.2009.24
15. Paula-Lima AC, Brito-Moreira J, Ferreira ST. Deregulation of excitatory neurotransmission underlying synapse failure in Alzheimer's disease. *J Neurochem*. (2013) 126:191–202. doi: 10.1111/jnc.12304
16. Wang R, Reddy PH. Role of glutamate and NMDA receptors in Alzheimer's disease. *J Alzheimers Dis*. (2017) 57:1041–8. doi: 10.3233/JAD-160763
17. Lipton SA. The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism. *Curr Alzheimer Res*. (2005) 2:155–65. doi: 10.2174/1567205053585846
18. McKeage K. Memantine: a review of its use in moderate to severe Alzheimer's disease. *CNS Drugs* (2009) 23:881–97. doi: 10.2165/11201020-000000000-00000
19. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* (2000) 47:351–4. doi: 10.1016/S0006-3223(99)00230-9
20. Zarate CA Jr, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry* (2006) 63:856–64. doi: 10.1001/archpsyc.63.8.856
21. Bak LK, Schousboe A, Waagepetersen HS. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem*. (2006) 98:641–53. doi: 10.1111/j.1471-4159.2006.03913.x
22. Walton HS, Dodd PR. Glutamate-glutamine cycling in Alzheimer's disease. *Neurochem Int*. (2007) 50:1052–66. doi: 10.1016/j.neuint.2006.10.007
23. Pomara N, Singh R, Deptula D, Chou JC, Schwartz MB, LeWitt PA. Glutamate and other CSF amino acids in Alzheimer's disease. *Am J Psychiatry* (1992) 149:251–4. doi: 10.1176/ajp.149.2.251
24. Jimenez-Jimenez FJ, Molina JA, Gomez P, Vargas C, de Bustos F, Benito-Leon J, et al. Neurotransmitter amino acids in cerebrospinal fluid of patients with Alzheimer's disease. *J Neural Transm*. (1998) 105:269–77. doi: 10.1007/s007020050073
25. Kaiser E, Schoenknecht P, Kassner S, Hildebrandt W, Kinscherf R, Schroeder J. Cerebrospinal fluid concentrations of functionally important amino acids and metabolic compounds in patients with mild cognitive impairment and Alzheimer's disease. *Neurodegener Dis*. (2010) 7:251–9. doi: 10.1159/000287953
26. Tohgi H, Abe T, Takahashi S, Kimura M. A selective reduction of excitatory amino acids in cerebrospinal fluid of patients with Alzheimer type dementia compared with vascular dementia of the Binswanger type. *Neurosci Lett*. (1992) 141:5–8. doi: 10.1016/0304-3940(92)90321-W
27. Martinez M, Frank A, Diez-Tejedor E, Hernanz A. Amino acid concentrations in cerebrospinal fluid and serum in Alzheimer's disease and vascular dementia. *J Neural Transm Park Dis Dement Sect*. (1993) 6:1–9. doi: 10.1007/BF02252617
28. Smith CC, Bowen DM, Francis PT, Snowden JS, Neary D. Putative amino acid transmitters in lumbar cerebrospinal fluid of patients with histologically verified Alzheimer's dementia. *J Neurol Neurosurg Psychiatry* (1985) 48:469–471. doi: 10.1136/jnnp.48.5.469
29. Degrell I, Hellsing K, Nagy E, Niklasson F. Amino acid concentrations in cerebrospinal fluid in presenile and senile dementia of Alzheimer type and multi-infarct dementia. *Arch Gerontol Geriatr*. (1989) 9:123–35. doi: 10.1016/0167-4943(89)90033-2
30. D'Aniello A, Fisher G, Migliaccio N, Cammisa G, D'Aniello E, Spinelli P. Amino acids and transaminases activity in ventricular CSF and in brain of normal and Alzheimer patients. *Neurosci Lett*. (2005) 388:49–53. doi: 10.1016/j.neulet.2005.06.030
31. Procter AW, Palmer AM, Francis PT, Lowe SL, Neary D, Murphy E, et al. Evidence of glutamatergic denervation and possible abnormal metabolism in Alzheimer's disease. *J Neurochem*. (1988) 50:790–802. doi: 10.1111/j.1471-4159.1988.tb02983.x
32. Levine J, Panchalingam K, Rapoport A, Gershon S, McClure RJ, Pettegrew JW. Increased cerebrospinal fluid glutamine levels in depressed patients. *Biol Psychiatry* (2000) 47:586–593. doi: 10.1016/S0006-3223(99)00284-X
33. Frye MA, Tsai GE, Huggins T, Coyle JT, Post RM. Low cerebrospinal fluid glutamate and glycine in refractory affective disorder. *Biol Psychiatry* (2007) 61:162–166. doi: 10.1016/j.biopsych.2006.01.024
34. Garakani A, Martinez JM, Yehuda R, Gorman JM. Cerebrospinal fluid levels of glutamate and corticotropin releasing hormone in major depression before and after treatment. *J Affect Disord*. (2013) 146:262–265. doi: 10.1016/j.jad.2012.06.037

35. Hashimoto K, Bruno D, Nierenberg J, Marmar CR, Zetterberg H, Blennow K, et al. Abnormality in glutamine-glutamate cycle in the cerebrospinal fluid of cognitively intact elderly individuals with major depressive disorder: a 3-year follow-up study. *Transl Psychiatry* (2016) 6:e744. doi: 10.1038/tp.2016.8
36. Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, et al. Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. *Neurology* (1999) 52:1555–62. doi: 10.1212/WNL.52.8.1555
37. Tabaraud F, Leman JP, Milor AM, Roussie JM, Barriere G, Tartary M, et al. Alzheimer CSF biomarkers in routine clinical setting. *Acta Neurol Scand*. (2012) 125:416–23. doi: 10.1111/j.1600-0404.2011.01592.x
38. Reis T, Brandao CO, Freire Coutinho ES, Engelhardt E, Laks J. Cerebrospinal fluid biomarkers in Alzheimer's disease and geriatric depression: preliminary findings from Brazil. *CNS Neurosci Ther*. (2012) 18:524–9. doi: 10.1111/j.1755-5949.2012.00311.x
39. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* (1993) 43:2412–4. doi: 10.1212/WNL.43.11.2412-a
40. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. (1975) 12:189–98. doi: 10.1016/0022-3956(75)90026-6
41. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorder, 4th ed*. Washington, DC: American Psychiatric Association (1994).
42. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* (1960) 23:56–62. doi: 10.1136/jnnp.23.1.56
43. Moreno DH, Moreno RA, Calil HM. A Brazilian experience of treatment-resistant depression. *In Clin Psychopharmacol.* (1994) 2:11–6. doi: 10.1097/00004850-199406002-00003
44. Maier W, Philipp M. Comparative analysis of observer depression scales. *Acta Psychiatr Scand*. (1985) 72:239–45. doi: 10.1111/j.1600-0447.1985.tb02601.x
45. WHO. *Mental Behavioral and Developmental Disorders. In International Statistical Classification of Diseases*. Geneva: WHO (1992).
46. Hashimoto A, Nishikawa T, Oka T, Takahashi K, Hayashi T. Determination of free amino acid enantiomers in rat brain and serum by high-performance liquid chromatography after derivatization with N-tert-butyloxycarbonyl-L-cysteine and o-phthaldialdehyde. *J Chromatogr*. (1992) 582:41–8. doi: 10.1016/0378-4347(92)80300-F
47. Calcia MA, Madeira C, Alheira FV, Silva TC, Tannos FM, Vargas-Lopes C, et al. Plasma levels of D-serine in Brazilian individuals with schizophrenia. *Schizophr Res*. (2012) 142:83–7. doi: 10.1016/j.schres.2012.09.014
48. Madeira C, Lourenco MV, Vargas-Lopes C, Suemoto CK, Brandão CO, Reis T, et al. D-serine levels in Alzheimer's disease: implications for novel biomarker development. *Transl Psychiatry* (2015) 5:e561. doi: 10.1038/tp.2015.52
49. Hashimoto K, Malchow B, Falkai P, Schmitt A. Glutamate modulators as potential therapeutic drugs in schizophrenia and affective disorders. *Eur Arch Psychiatry Clin Neurosci.* (2013) 263:367. doi: 10.1007/s00406-013-0399-y
50. Kochhann R, Varela JS, Lisboa CS de M, Chaves MLF. The Mini Mental State Examination: Review of cutoff points adjusted for schooling in a large Southern Brazilian sample. *Dement Neuropsychol.* (2010) 4:35–41. doi: 10.1590/S1980-57642010DN40100006
51. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* (1984) 34:939–44. doi: 10.1212/WNL.34.7.939
52. Jack CR Jr, Albert MS, Knopman DS, McKhann GM, Sperling RA, Carrillo MC, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. (2011) 7:257–62. doi: 10.1016/j.jalz.2011.03.004
53. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. (2011) 7:263–9. doi: 10.1016/j.jalz.2011.03.005
54. Hurley LL, Tizabi Y. Neuroinflammation, neurodegeneration, and depression. *Neurotox Res*. (2013) 23:131–44. doi: 10.1007/s12640-012-9348-1
55. Leonard BE. The concept of depression as a dysfunction of the immune system. *Curr Immunol Rev*. (2010) 6:205–12. doi: 10.2174/157339510791823835
56. Hermida AP, McDonald WM, Steenland K, Levey A. The association between late-life depression, mild cognitive impairment and dementia: is inflammation the missing link? *Expert Rev Neurother*. (2012) 12:1339–50. doi: 10.1586/ern.12.127
57. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* (2009) 65:732–41. doi: 10.1016/j.biopsych.2008.11.029
58. Niciu MJ, Ionescu DF, Richards EM, Zarate CA. Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *J Neural Transm.* (2014) 121:907–24. doi: 10.1007/s00702-013-1130-x
59. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol*. (2016) 16:22–34. doi: 10.1038/nri.2015.5
60. Heser K, Tebarth F, Wiese B, Eisele M, Bickel H, Kohler M, et al. Age of major depression onset, depressive symptoms, and risk for subsequent dementia: results of the German Study on Ageing, Cognition, and Dementia in Primary Care Patients (AgeCoDe). *Psychol Med.* (2012) 43:1597–610. doi: 10.1017/S0033291712002449
61. Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. *Behav Brain Res*. (2003) 140:1–47. doi: 10.1016/S0166-4328(02)00272-3
62. Machado-Vieira R, Salvadore G, Ibrahim LA, Diaz-Granados N, Zarate CA Jr. Targeting glutamatergic signaling for the development of novel therapeutics for mood disorders. *Curr Pharm Des*. (2009) 15:1595–611. doi: 10.2174/138161209788168010
63. Selkoe DJ. Alzheimer's disease. *Cold Spring Harb Perspect Biol*. (2011) 3:a004457.
64. Ferreira ST, Lourenco MV, Oliveira MM, De Felice FG. Soluble amyloid-β oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. *Front Cell Neurosci.* (2015) 9:191. doi: 10.3389/fncel.2015.00191
65. Brito-Moreira J, Paula-Lima AC, Bomfim TR, Oliveira FB, Sepulveda FJ, De Mello FG, et al. Abeta oligomers induce glutamate release from hippocampal neurons. *Curr Alzheimer Res*. (2011) 8:552–62. doi: 10.2174/156720511796391917
66. Figueiredo CP, Clarke JR, Ledo JH, Ribeiro FC, Costa CV, Melo HM, et al. Memantine rescues transient cognitive impairment caused by high-molecular-weight aβ oligomers but not the persistent impairment induced by low-molecular-weight oligomers. *J Neurosci.* (2013) 33:9626–34. doi: 10.1523/JNEUROSCI.0482-13.2013
67. Lourenco MV, Clarke JR, Frozza RL, Bomfim TR, Forny-Germano L, Batista AF, et al. TNF-α mediates PKR-dependent memory impairment and brain IRS-1 inhibition induced by Alzheimer's β-amyloid oligomers in mice and monkeys. *Cell Metab.* (2013) 18:831–43. doi: 10.1016/j.cmet.2013.11.002

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 06 November 2018
doi: 10.3389/fpsyt.2018.00544

Astrocytic Regulation of Glutamate Transmission in Schizophrenia

Yu-Ying Mei[1,2], *Dong Chuan Wu*[1,2] *and Ning Zhou*[1,2]*

[1] *Translational Medicine Research Center, China Medical University Hospital, Taichung, Taiwan,* [2] *Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan*

According to the glutamate hypothesis of schizophrenia, the abnormality of glutamate transmission induced by hypofunction of NMDA receptors (NMDARs) is causally associated with the positive and negative symptoms of schizophrenia. However, the underlying mechanisms responsible for the changes in glutamate transmission in schizophrenia are not fully understood. Astrocytes, the major regulatory glia in the brain, modulate not only glutamate metabolism but also glutamate transmission. Here we review the recent progress in understanding the role of astrocytes in schizophrenia. We focus on the astrocytic mechanisms of (i) glutamate synthesis via the glutamate-glutamine cycle, (ii) glutamate clearance by excitatory amino acid transporters (EAATs), (iii) D-serine release to activate NMDARs, and (iv) glutamatergic target engagement biomarkers. Abnormality in these processes is highly correlated with schizophrenia phenotypes. These findings will shed light upon further investigation of pathogenesis as well as improvement of biomarkers and therapies for schizophrenia.

Keywords: schizophrenia, glutamate, NMDA receptors, excitatory amino acid transporters, glutamate-glutamine cycle, D-serine

OPEN ACCESS

Edited by:
Kenji Hashimoto,
Chiba University, Japan

Reviewed by:
Jennifer C. Felger,
Emory University, United States
Kurt Leroy Hoffman,
Autonomous University of Tlaxcala, Mexico

***Correspondence:**
Ning Zhou
ningzhou.nrsc@gmail.com

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *12 July 2018*
Accepted: *12 October 2018*
Published: *06 November 2018*

Citation:
Mei Y-Y, Wu DC and Zhou N (2018) Astrocytic Regulation of Glutamate Transmission in Schizophrenia. Front. Psychiatry 9:544. doi: 10.3389/fpsyt.2018.00544

INTRODUCTION

Dysregulation of glutamatergic neurotransmission is critically implicated in the pathophysiology of psychotic disorders. In the central nervous system (CNS), glutamate is the principal excitatory neurotransmitter and mediates the fast excitatory transmission by activation of ionotropic glutamate receptors, including AMPA, kainate, and NMDA receptors (NMDARs). Astrocytes affect glutamatergic transmission in several important ways, including glutamate biosynthesis, glutamate-glutamine cycle, glutamate uptake, and releasing glutamate and D-serine as gliotransmitters. This review focuses on recent advances in astrocyte-mediated dysregulation of glutamate transmission in schizophrenia.

THE GLUTAMATE HYPOTHESIS OF SCHIZOPHRENIA

The "glutamate hypothesis of schizophrenia" proposes that schizophrenia symptoms and cognitive impairment are due to hypofunction of NMDARs and excessive glutamate release, especially in brain areas including prefrontal cortex and hippocampus (1). This theory was initiated from the observation that NMDAR antagonists, like phencyclidine and ketamine, could evoke negative symptoms and cognitive dysfunction resembling schizophrenic phenotypes in healthy subjects (2). In schizophrenic subjects, subanesthetic doses of ketamine could exacerbate psychotic and cognitive symptoms (3, 4). Interestingly, in encephalitis patients, the first-episode psychosis is associated with the presence of anti-NMDAR antibodies that could cause a reduction of

surface expression of NMDARs (5, 6). In animal studies, suppression of NMDAR function by pharmacological or genetic approaches leads to schizophrenia-like behaviors (7, 8). Schizophrenia is also associated with dysregulation of some genes and/or proteins involved in glutamate transmission (9). Genome-wide association studies have reported that the NMDAR subunits encoding genes, GRIN2A and GRIN2B, are schizophrenia-related genes (9, 10). Two *de novo* mutations in GRIN2A were found in sporadic schizophrenia patients (11). The single nucleotide polymorphisms of genes related to D-serine synthesis and metabolism, such as genes encoding serine racemase (SR), D-Amino Acid Oxidase (DAAO), and the DAAO activator G72, are also associated with schizophrenia (12).

The hypofunction of NMDAR causes excessive glutamate release, hyper-glutamatergic functions, and hypermetabolism (13). However, it remains largely unknown how NMDAR hypofunction leads to elevated glutamate levels and over-activation of non-NMDA glutamate receptors. One possibility is that interneurons are more sensitive to NMDAR blockade than principal glutamatergic neurons, and interneuron inhibition leads to an overall effect of disinhibition and hyperexcitation of glutamatergic output (**Figure 1**) (1, 14, 15). The increased glutamate levels have been documented by brain imaging studies and the source of excessive glutamate is mainly from presynaptic release (16). This notion was strengthened by the effect of drugs that specifically reduce presynaptic glutamate release, like the mGluR2/3 agonists, which significantly reverse ketamine-evoked glutamate elevation and cerebral blood volume increase in the hippocampus (13). High extracellular glutamate levels lead to hypermetabolism, structural disorganization, and eventually hippocampal volume reduction, and these dysfunctions are correlated with disease progression from prodromal symptoms to psychosis (17). Nevertheless, many mechanisms that cause an overall increase in glutamate tone could contribute synergistically to schizophrenia pathogenesis.

SYNAPTIC AND EXTRASYNAPTIC NMDARS

NMDARs are distributed at both synaptic and extrasynaptic sites with different subunit compositions. Their activation requires the binding of both glutamate and D-serine/glycine as a co-agonist. Synaptic NMDARs are activated by presynaptic glutamate release at low frequency, while intensive synaptic activation could lead to glutamate spillover and activation of extrasynaptic NMDARs (18). Synaptic NMDARs are important for synaptic plasticity and learning and memory, which is largely controlled by D-serine concentration within the synaptic cleft depending on astrocyte coverage and function (19, 20). Moreover, extrasynaptic NMDARs are in close proximity to synapse-enveloping astrocytes and are a preferential target for astrocyte-released glutamate, D-serine, and glycine (**Figure 2**). Activation of extrasynaptic NMDARs could generate two forms of responses. First, ambient glutamate could produce a tonic NMDAR current, whose amplitude is directly modulated by glial EAATs (21, 22). The tonic NMDAR currents contribute to neuronal excitability and their existence has been identified in multiple neuron types in the prefrontal cortex, hippocampus, and cerebellum (23–25). Second, activation of extrasynaptic NMDARs could produce "slow inward currents" (SICs), which are infrequent phasic events characterized by slow activation and decay kinetics, large amplitude, and insensitivity to sodium channel blockers. It is believed that SICs are due to astrocyte-originated glutamate and the co-occurrence of SICs in adjacent neurons could lead to synchronized neuronal activity (26). It is also possible that SICs are generated by the phasic release of D-serine/glycine, which potentiates extrasynaptic NMDARs that are already tonically activated by low concentrations of ambient glutamate (18). While accumulating evidence has demonstrated a critical role of deficient synaptic NMDARs in schizophrenia (27, 28), alterations in the expression and function of extrasynaptic NMDARs are less understood. It was recently reported that the tonic NMDAR conductance was significantly larger and more sensitive to NMDAR blockers in interneurons compared with pyramidal neurons in the hippocampus (29). However, it remains to be determined whether extrasynaptic NMDAR deficiency occurs under schizophrenic conditions.

ASTROCYTES AND GLUTAMATE TRANSMISSION

Regulation of glutamate transmission could occur at several steps, including synthesis, transportation, release, and clearance of glutamate, as well as activation of glutamate receptors by endogenous agonists and co-agonists. **Figure 2** summarizes some key mechanisms underlying the impact of astrocytes in glutamate transmission in the CNS. Astrocytes are the primary locus for the biosynthesis of glutamate from glucose. Through the tricarboxylic acid cycle, the glycolysis product pyruvate is converted into α-ketoglutarate, which is then catalyzed into glutamate by aspartate aminotransferase (30). Glutamate is catalyzed by glutamine synthetase (GS), which is exclusively expressed in astrocytes in the brain, to form glutamine that is further transferred to neurons. In glutamatergic neurons, glutamate is converted from glutamine by phosphate-activated glutaminase (30). After packaged into synaptic vesicles, glutamate is released as the neurotransmitter to active postsynaptic AMPA/kainate receptors to mediate fast excitatory synaptic transmission, and to activate NMDARs to depolarize the postsynaptic membrane potential and to allow Ca^{2+} influx. The clearance of excessive glutamate from the synaptic cleft occurs rapidly, largely due to the function of four subtypes of EAATs (EAAT1-4), among which the EAAT1 (also termed glutamate-aspartate transporter, GLAST) and EAAT2 (also termed glutamate transporter 1, GLT-1) are mainly expressed in astrocytes (31). Once glutamate is taken up into astrocytes, it is converted into glutamine and re-enters the glutamate-glutamine cycle. Besides their key roles in the biosynthesis of glutamate and maintenance of the glutamate-glutamine cycle, astrocytes can directly release glutamate as a gliotransmitters by either Ca^{2+}-dependent vesicular exocytosis or by non-vesicular release (32, 33). Astrocytes are also a major source of glycine

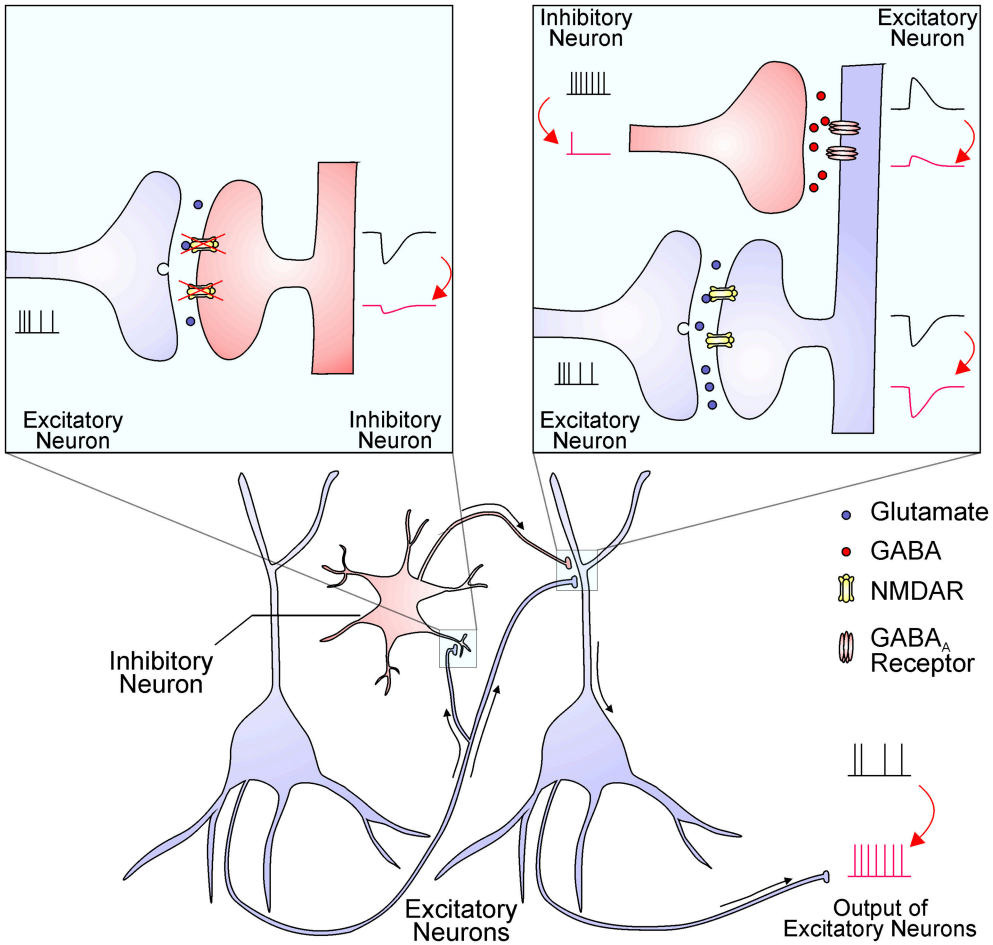

FIGURE 1 | The NMDAR hypofunction hypothesis proposes that NMDARs in inhibitory interneurons are preferentially diminished in schizophrenia. The reduced NMDAR function in interneurons (the left inset) results in decreased excitatory postsynaptic currents, which in turn decrease interneuron output (the right inset). The reduced GABA release from interneuron terminals leads to smaller inhibitory postsynaptic responses and thereby a disinhibition of postsynaptic excitatory neurons (the right inset). This finally leads to an enhanced output of excitatory neurons, resulting in excessive glutamate release and increased activation of non-NMDA glutamate receptors. This process might be enhanced by NMDAR antagonists and ameliorated by mGluR2/3 agonists.

and D-serine to potentiate NMDAR responses. Glycine is stored in astrocytes with high concentrations (3–6 mM) and can be released via the reversed operation of glycine transporters and other Ca^{2+}-independent pathways (34–36). The synthesis of D-serine is initiated from the glycolytic intermediate metabolite 3-phosphoglycerate, which is synthesized into L-serine in several steps. D-serine is converted from L-serine by the action of SR and is released by Ca^{2+}-dependent and/or -independent machineries from astrocytes (19, 37) [but see (38)]. Notably, these above processes might interact with one another; for example, the increased glutamate synthesis leads to elevated glutamate release (39), and activation of glutamate transports could evoke rapid glutamine release as a feedback mechanism (40).

EAATS

The efficiency of glutamate clearance directly affects extracellular resting glutamate levels and kinetics of glutamate-mediated synaptic responses. Among the total four subtypes of brain-expressed EAATs, EAAT1, and EAAT2 are mainly expressed in astrocytes, and EAAT2 is the dominant transporter in adults and is responsible for about 90% of total glutamate uptake (41, 42). The EAATs are mainly responsible for clearing synaptic glutamate and shaping postsynaptic responses. Deficits in EAAT functions cause the persistently increased glutamate levels, which represent one of the most remarkable changes in the schizophrenic brain. Accumulating evidence has reported abnormal mRNA and/or protein expression levels of EAATs in the prefrontal cortex, hippocampus, and anterior cingulate cortex in the postmortem tissue from schizophrenia patients. While many studies have revealed an overall reduction of EAAT2 levels in schizophrenia, some other studies reported unchanged or increased EAAT2 levels in certain brain regions [for review see (43)]. In a recent study using the laser-capture microdissection technique to separately harvest glutamatergic relay neurons and astrocytes from the mediodorsal nucleus of

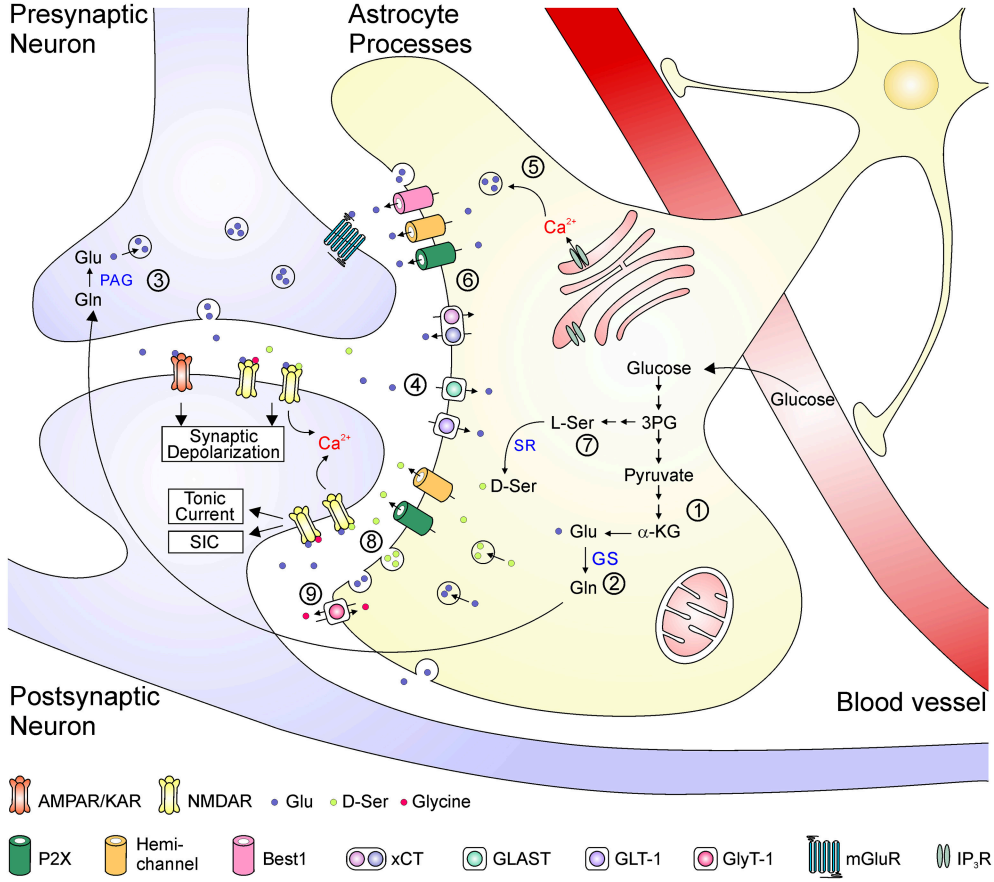

FIGURE 2 | Schematic depiction of the astrocyte-related mechanisms in the metabolism, transport, uptake of glutamate and the regulation of glutamate transmission. Glutamate is synthesized *de novo* from α-KG via the TCA cycle (1). In the glutamate-glutamine cycle (2–4), glutamate is metabolized to glutamine by GS in the astrocyte and then transported to the neuron (2). Neuronal PAG catalyzes the conversion from glutamine into glutamate, which is released as the transmitter from presynaptic terminal (3). The synaptically released glutamate is rapidly uptake by EAATs back into the astrocytes (4). Glutamate can be released as a gliotransmitters by either Ca^{2+}-dependent vesicular release (5) or efflux from transporters or large-conductance ion channels (6). D-serine is converted from L-serine, which is synthesized *de novo* from 3-phosphoglycerate in astrocytes (7). D-serine can be released via either vesicular or non-vesicular mechanisms and activate neuronal NMDA receptors (8). Glycine is transported across astrocytic membrane via glycine receptors (9). 3PG, 3-phosphoglycerate; α-KG, α-ketoglutarate; Glu, glutamate; Gln, glutamine; GS, glutamine synthetase; L-Ser, L-serine; D-Ser, D-serine; PAG, phosphate-activated glutaminase; SR, serine racemase; ASCT, alanine-serine-cysteine transporter; xCT, cystine-glutamate antiporter; GLAST, glutamate aspartate transporter; GLT-1, glutamate transporter 1; GlyT-1, glycine transporter 1; mGluR, metabotropic glutamate receptor; IP3R, inositol trisphosphate receptor.

thalamus, it was reported that changes of EAAT levels could be cell-specific: mRNA expression of EAAT1 was decreased in astrocytes, whereas EAAT2 was increased in excitatory relay neurons in schizophrenia (44). The upregulation of EAATs in neurons is possibly due to a compensatory response to the reduced EAAT levels in astrocytes. Notably, the mRNA levels of EAAT2b were remarkably increased in anterior cingulate cortex pyramidal neurons and thalamus relay neurons in schizophrenia (44, 45). EAAT2b is a splicing variant of EAAT2. It contains the PDZ domain-binding motif and possibly interacts with neuronal scaffolding proteins like PSD-95 (45–47). The EAAT2b proteins are localized predominantly at the cell surface and their distribution can be regulated by intracellular Ca^{2+} and CaMKII (48), suggesting that EAAT2b might exhibit neuronal specific functions.

The abnormal EAAT levels are highly associated with schizophrenia phenotypes. Mice lacking EAAT1 displayed schizophrenia-like behavioral changes: they showed higher locomotor activity in the novel environment but not in the home cage, and had increased sensitivity to the locomotor hyperactivity induced by NMDA antagonists (49). The locomotor hyperactivity of EAAT1 knockout mice was reversed by either the antipsychotic haloperidol or the mGlu2/3 agonist LY379268 (49). These mice also exhibited abnormalities in behavioral tests that measure the negative and cognitive symptoms of schizophrenia, including poor nesting behaviors, lesser preference for novel social stimulus albeit normal overall social interaction, a significant reduction in acoustic startle amplitude, and impaired learning in an instrumental visual discrimination task, suggesting that EAAT1 dysfunction could generate certain behavioral changes

resembling schizophrenia phenotypes (49, 50). Knockout of EAAT2 caused lethal spontaneous seizures, high susceptibility to brain injury, and short life-span in the homozygous mice (51). The heterozygous EAAT2 knockout mice had less severe abnormalities, and they exhibited increased locomotion activity in a new environment and greater freezing responses in the fear-conditioning test compared to the wildtype (52). The EAAT1/2 double knockout mice showed multiple brain defects resembling developmental defects found in schizophrenia (53). In a recent study, the inducible EAAT2 knockout mice were generated in a temporal controlled, cell-specific manner. The study demonstrated that knockout during adolescence caused 60–80% reduction of EAAT2 selectively in astrocytes, and the animals showed no lethal seizures or neuronal loss. Interestingly, these animals exhibited pathological repetitive behaviors, like excessive self-grooming and tic-like head shakes, whereas they had no obvious anxiety or social abnormality (54).

It remains unknown how EAAT abnormality leads to schizophrenia-relevant behaviors. In hippocampus, pharmacological inhibition of EAAT functions increased the peak amplitude of NMDAR-mediated EPSCs and prolonged their decay kinetics, but had no changes in AMPA receptor-mediated EPSCs, possibly due to the fast desensitization of AMPA receptor currents (55). It has been generally considered that EAATs could limit glutamate spillover to neighboring synapses and enhance synapse independence, which is related to synaptic plasticity as well as learning and memory (31). Reduced expression of glial EAATs will not only impair these important physiological functions but also increase the susceptibility of the brain to injury and cell death (51). Neuronal EAATs might have much lower efficiency than the astrocytic transports: glutamate is quickly converted into glutamine by GS, resulting in low free glutamate concentrations in astrocytes; whereas neurons are liable to accumulate intracellular glutamate concentration and thereby lead to poor operation of neuronal EAATs. Moreover, the action of EAATs will produce a non-stoichiometrically coupled anion current (56, 57), which contributes to the regulation of neuronal membrane potentials and transmitter release (58). In the zebrafish photoreceptor synapse, the EAAT2b isoform mediates a large-conductance Cl^- current, potentially capable of affecting resting membrane potentials (59). Nevertheless, these mechanisms remain to be determined in schizophrenia and may vary depending on the brain region and disease progression.

THE GLUTAMATE-GLUTAMINE CYCLE

Schizophrenia is associated with the abnormal glutamate-glutamine cycle. Altered levels of brain metabolites including glutamate, GABA, and glutamine have been well-documented in human studies and these alterations can be detected in different brain regions and the cerebrospinal fluid (CSF) (60). Researchers using proton magnetic resonance spectroscopy (^{1}H-MRS) have reported abnormal glutamine and/or glutamate levels (some studies report glutamate+glutamine levels in combination depending on the ^{1}H-MRS approach and field strength), especially in the brain regions involving the medial temporal lobe (including hippocampus), basal ganglia, and thalamus of schizophrenia patients (61). An elevation of the glutamate+glutamine level was also observed in hippocampus of healthy humans receiving ketamine administration (62). The CSF glutamine/glutamate ratio is higher in the first-episode patients compared with normal controls (63). GS, the key enzyme involved in the glutamate-glutamine cycle, exhibits lower protein levels in spite of increased expression of mRNA in schizophrenia; but the results were less consistent among studies [for review see (43)]. Elevated glutamine levels are also directly related to psychotic symptoms and neuropsychological tests (64, 65). Although changed glutamine levels are supposedly related to functional alterations of astrocytes, it is notable that the ^{1}H-MRS and CSF data cannot provide a precise measure of intracellular vs. extracellular substrates and further evidence is needed to establish the causality.

Disturbance of the glutamate-glutamine cycle and down-regulation of GS have also been reported in human epilepsy (66, 67). Reduced GS activity will build up intracellular glutamate in astrocytes, leading to reduced uptake capacities of EAATs. Moreover, glutamine produced by astrocytic GS is also one of the major sources for maintaining synaptic vesicle content of GABA in inhibitory interneurons (68). Pharmacological inhibition of GS reduced inhibitory postsynaptic currents in hippocampal pyramidal neurons and was reversed by exogenous application of glutamine (68). Similar dysfunction of the inhibitory synaptic response was also found in neurons near reactive astrocytes, which showed profound decreases in GS expression levels (69). These effects increased network hyperexcitability and produced spontaneous recurrent seizures (70). The loss of interneurons and deficient cortical GABA synthesis are also one of the most robust pathologies of schizophrenia (71–73). The reduced parvalbumin interneurons are associated with impaired oscillatory activity in hippocampus and imbalance of cortical excitation-inhibition (72, 74). Impaired parvalbumin interneuron activity by selective deletion of ErbB4 or dopamine D2 receptors produced schizophrenia-like behaviors in mice (75, 76). Dysfunction of neuron-astrocyte interaction has been suggested in schizophrenia models (77). However, it remains unclear whether there is any causative relationship between the dysfunction of the glutamate-glutamine cycle and interneuron deficiency.

D-SERINE

D-serine plays an important role in the dysregulation of NMDAR functions in schizophrenia (78). This was supported by several lines of evidence, including the reduced serum and CSF D-serine levels (79, 80), a genetic association of SR and DAAO (the enzyme responsible for the degradation of D-serine) polymorphisms in schizophrenia patients (12, 81), the reversal effects of D-serine on schizophrenia-like behaviors in animals (82), and the beneficial effects of clinical D-serine-targeting therapies in patients (83, 84).

Recent studies revealed that the abnormality in astrocyte-released D-serine plays a critical role in the DISC1-associated

pathological processes. The DISC1 gene was identified from patients with familial mental disorders and has been considered a critical susceptibility gene for schizophrenia (85, 86). The density of DISC1-expressing astrocytes is largely reduced in the dentate gyrus of hippocampus in schizophrenia patients compared with healthy controls (87). Selective expression of mutant DISC1 in astrocytes decreased protein levels of SR by increasing its ubiquitination (88). Astrocytes expressing mutant DISC1 caused less elaborated dendritic arborization and decreased the density of excitatory synapses in co-cultured normal neurons (89). The downregulation of SR resulted in a significant decline in D-serine levels, an enhanced locomotor activity, and impaired responses to pre-pulse inhibition in the mutant mice (88). Mice carrying the astrocytic specific mutation of DISC1 also exhibited increased anxiety, attenuated social interaction and preference for social novelty, and impaired cognitive behaviors (90). These behavioral abnormalities in DISC1 mutant mice were reversed by treatments of D-serine (88, 90), indicating a strong correlation with NMDAR hypofunction.

The α7 subunit-containing nicotinic acetylcholine receptor (α7 nAChR) has been considered a strong genetic contribution to schizophrenia (91). It has been reported that activation of α7 nAChR contributes to dopamine release and induction of long-term potentiation (91). Interestingly, α7 nAChRs are expressed not only in neurons but also in astrocytes (92–94). ACh could induce the intracellular Ca^{2+} elevation in astrocytes by activation of α7 nAChRs and facilitate the synthesis and release of D-serine (94–97). Papouin et al. recently discovered that astrocytic α7 nAChRs activation could drive the vesicular release of D-serine from astrocytes to generate a wakefulness-dependent D-serine oscillation *in z* (98). The wakefulness and activity but not the circadian rhythms of the animal promote D-serine fluctuations over the 24 h period, during which D-serine was accumulated during the wakefulness and declined during sleep. Such an oscillation of the D-serine level is associated with NMDAR activity and the learning and memory behavior of the animal, consistent with the involvement of NMDARs in the cognitive deficits in schizophrenia. Astrocytes appear to play a central role since D-serine accumulation during the wakefulness is impaired by disruption of astrocytic vesicular exocytosis machinery or by selective deletion of α7 nAChRs from astrocytes rather than neurons. More importantly, EVP-6124, an α7 nAChR modulator tested in schizophrenia clinical trials, is able to promote D-serine release and enhance NMDAR activity (98).

POTENTIAL BIOMARKERS

Several types of translational biomarkers have been developed to assess prognosis and monitor disease progression and treatment in schizophrenia. First, functional magnetic imaging (fMRI) blood-oxygenation-level dependent response (BOLD) has been applied to test brain physiology following acute NMDAR antagonist administration. NMDAR antagonists evoke robust changes in relative cerebral blood volume and local metabolism, which is largely due to the energy consumption for increased glutamate release and uptake (13, 99). The second approach applies ^{1}H-MRS to measure glutamine and/or glutamate levels in the brain (16, 100). As described above, abnormal glutamine and/or glutamate indices were found in brain regions of individuals with schizophrenia compared with healthy volunteers (61). The third method utilizes task-based fMRI to evaluate BOLD responses in hippocampus and dorsolateral prefrontal cortex (101). The most adapted task is item-specific encoding task, which is designed to assess contributions of specific encoding and retrieval processes to episodic memory (102). Schizophrenia patients with more severe negative symptoms exhibited poor BOLD responses during the encoding and retrieval of episodic memory (101, 103). And fourth, positron emission tomography (PET) technique has been used to evaluate glutamatergic, GABAergic, and monoamine neurotransmission in various brain functions and have established the association between these responses and cognitive functions in schizophrenia (104, 105). Finally, the severity of schizophrenia symptoms is associated with abnormal serum levels of several glutamate transmission related factors, including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), D-serine, G72, and multiple inflammatory factors (106–108). A recent clinical study by Javitt et al. compared the first three types of glutamatergic target-engagement biomarkers in healthy volunteers receiving ketamine or placebo infusions (109). The data revealed a significant increase in the NMDAR antagonist-evoked BOLD responses. The ^{1}H-MRS results showed a smaller but significant change in glutamate+glutamine levels. However, the data from task-based fMRI failed to yield a significant difference between the ketamine and placebo group (109). These observations indicate the fMRI BOLD as a particularly relevant biomarker for NMDAR antagonist-induced responses. The potential application of one or combined biomarkers for more refined prognostic classification of schizophrenia remains to be further assessed.

FUTURE TREATMENTS

Based on the glutamate hypothesis, many drug targets have been proposed and some are under investigation. For example, drugs targeting the co-agonist binding site of NMDARs are of the primary interest [for review see (110)]. Direct enhancement of NMDAR functions with glycine and D-serine has been used in multiple clinical trials, among which one recent study showed improved negative symptoms in individuals at clinical high risk of schizophrenia (111). GlyT1 inhibitors, like sarcosine or Bitopertin, which are supposed to increase synaptic glycine levels, have been found effective in several trials (112). Other treatments to increase D-serine/glycine levels, like the DAAO inhibitor sodium benzoate, have demonstrated beneficial effects as an add-on drug (83, 84). The mGluR2/3 agonists have been shown to inhibit excessive glutamate release in animal studies, and the tested drug LY2140023 has produced significant improvement in patients (113). Moreover, the α7 nAChR has attracted much attention; several drugs targeting this receptor are under clinical investigation (110). Besides these, more specific regulation of certain proteins in the glutamate transmission, such as selective

NMDAR subtypes or EAAT isoforms, might represent potential targets for future drug development.

CONCLUSIONS

The glutamate hypothesis has substantially advanced our understanding of the pathogenesis of schizophrenia. Although glutamate transmission is mediated by ionotropic glutamate receptors and involves other pre- and post-synaptic components at excitatory synapses, astrocytes regulate glutamate metabolism and shape glutamate transmission in several important aspects. This review has focused on some recent studies that explicitly report the important role of glutamate uptake, glutamate-glutamine cycle, and astrocyte-derived D-serine in the etiology of schizophrenia. Neuroimaging and neurochemical biomarkers based on glutamate transmission and metabolism have also demonstrated translational utility. Further understanding of neuron-astrocyte interaction during these processes will be critical to the development of diagnostic and therapeutic avenues for schizophrenia and psychotic disorders.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

This study was supported by grants to NZ and DW from the Ministry of Science and Technology (MOST 107-2320-B-039-060-MY3 and MOST 107-2320-B-039-061-MY3) and China Medical University Hospital (DMR-106-113).

REFERENCES

1. Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* (2012) 37:4–15. doi: 10.1038/npp.2011.181
2. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* (1994) 51:199–214. doi: 10.1001/archpsyc.1994.03950030035004
3. Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, et al. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology* (1997) 17:141–50. doi: 10.1016/S0893-133X(97)00036-5
4. Lahti AC, Koffel B, LaPorte D, Tamminga CA. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* (1995) 13:9–19. doi: 10.1016/0893-133X(94)00131-I
5. Lennox BR, Palmer-Cooper EC, Pollak T, Hainsworth J, Marks J, Jacobson L, et al. Prevalence and clinical characteristics of serum neuronal cell surface antibodies in first-episode psychosis: a case-control study. *Lancet Psychiatry* (2017) 4:42–8. doi: 10.1016/S2215-0366(16)30375-3
6. Moscato EH, Peng X, Jain A, Parsons TD, Dalmau J, Balice-Gordon RJ. Acute mechanisms underlying antibody effects in anti-N-methyl-D-aspartate receptor encephalitis. *Ann Neurol.* (2014) 76:108–19. doi: 10.1002/ana.24195
7. Jones CA, Watson DJ, Fone KC. Animal models of schizophrenia. *Br J Pharmacol.* (2011) 164:1162–94. doi: 10.1111/j.1476-5381.2011.01386.x
8. Ramsey AJ. NR1 knockdown mice as a representative model of the glutamate hypothesis of schizophrenia. *Prog Brain Res.* (2009) 179:51–8. doi: 10.1016/S0079-6123(09)17906-2
9. Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, et al. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet.* (2008) 40:827–34. doi: 10.1038/ng.171
10. Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* (2014) 511:421–7. doi: 10.1038/nature13595
11. Tarabeux J, Kebir O, Gauthier J, Hamdan FF, Xiong L, Piton A, et al. Rare mutations in N-methyl-D-aspartate glutamate receptors in autism spectrum disorders and schizophrenia. *Transl Psychiatry* (2011) 1:e55. doi: 10.1038/tp.2011.52
12. Boks MP, Rietkerk T, van de Beek MH, Sommer IE, de Koning TJ, Kahn RS. Reviewing the role of the genes G72 and DAAO in glutamate neurotransmission in schizophrenia. *Eur Neuropsychopharmacol.* (2007) 17:567–72. doi: 10.1016/j.euroneuro.2006.12.003
13. Schobel SA, Chaudhury NH, Khan UA, Paniagua B, Styner MA, Asllani I, et al. Imaging patients with psychosis and a mouse model establishes a spreading pattern of hippocampal dysfunction and implicates glutamate as a driver. *Neuron* (2013) 78:81–93. doi: 10.1016/j.neuron.2013.02.011
14. Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J Neurosci.* (2007) 27:11496–500. doi: 10.1523/JNEUROSCI.2213-07.2007
15. Maccaferri G, Dingledine R. Control of feedforward dendritic inhibition by NMDA receptor-dependent spike timing in hippocampal interneurons. *J Neurosci.* (2002) 22:5462–72. doi: 10.1523/JNEUROSCI.22-13-05462.2002
16. Poels EM, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, et al. Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. *Mol Psychiatry* (2014) 19:20–9. doi: 10.1038/mp.2013.136
17. Schobel SA, Lewandowski NM, Corcoran CM, Moore H, Brown T, Malaspina D, et al. Differential targeting of the CA1 subfield of the hippocampal formation by schizophrenia and related psychotic disorders. *Arch Gen Psychiatry* (2009) 66:938–46. doi: 10.1001/archgenpsychiatry.2009.115
18. Papouin T, Oliet SH. Organization, control and function of extrasynaptic NMDA receptors. *Philos Trans R Soc Lond B Biol Sci.* (2014) 369:20130601. doi: 10.1098/rstb.2013.0601
19. Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, et al. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* (2006) 125:775–84. doi: 10.1016/j.cell.2006.02.051
20. Henneberger C, Papouin T, Oliet SH, Rusakov DA. Long-term potentiation depends on release of D-serine from astrocytes. *Nature* (2010) 463:232–6. doi: 10.1038/nature08673
21. Le Meur K, Galante M, Angulo MC, Audinat E. Tonic activation of NMDA receptors by ambient glutamate of non-synaptic origin in the rat hippocampus. *J Physiol.* (2007) 580(Pt 2):373–83. doi: 10.1113/jphysiol.2006.123570
22. Fleming TM, Scott V, Naskar K, Joe N, Brown CH, Stern JE. State-dependent changes in astrocyte regulation of extrasynaptic NMDA receptor signalling in neurosecretory neurons. *J Physiol.* (2011) 589(Pt 16):3929–41. doi: 10.1113/jphysiol.2011.207340
23. Povysheva NV, Johnson JW. Tonic NMDA receptor-mediated current in prefrontal cortical pyramidal cells and fast-spiking interneurons. *J Neurophysiol.* (2012) 107:2232–43. doi: 10.1152/jn.01017.2011
24. Rossi DJ, Slater NT. The developmental onset of NMDA receptor-channel activity during neuronal migration. *Neuropharmacology* (1993) 32:1239–48. doi: 10.1016/0028-3908(93)90018-X
25. Zhang M, Biancardi VC, Stern JE. An increased extrasynaptic NMDA tone inhibits A-type K(+) current and increases excitability of hypothalamic

neurosecretory neurons in hypertensive rats. *J Physiol.* (2017) 595:4647–61. doi: 10.1113/JP274327
26. Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron* (2004) 43:729–43. doi: 10.1016/j.neuron.2004.08.011
27. Balu DT. The NMDA receptor and schizophrenia: from pathophysiology to treatment. *Adv Pharmacol.* (2016) 76:351–82. doi: 10.1016/bs.apha.2016.01.006
28. Banerjee A, Wang HY, Borgmann-Winter KE, MacDonald ML, Kaprielian H, Stucky A, et al. Src kinase as a mediator of convergent molecular abnormalities leading to NMDAR hypoactivity in schizophrenia. *Mol Psychiatry* (2015) 20:1091–100. doi: 10.1038/mp.2014.115
29. Riebe I, Seth H, Culley G, Dosa Z, Radi S, Strand K, et al. Tonically active NMDA receptors–a signalling mechanism critical for interneuronal excitability in the CA1 stratum radiatum. *Eur J Neurosci.* (2016) 43:169–78. doi: 10.1111/ejn.13128
30. Hertz L, Rothman DL. Glucose, lactate, beta-hydroxybutyrate, acetate, GABA, and succinate as Substrates for synthesis of glutamate and GABA in the glutamine-glutamate/GABA Cycle. *Adv Neurobiol.* (2016) 13:9–42. doi: 10.1007/978-3-319-45096-4_2
31. Tzingounis AV, Wadiche JI. Glutamate transporters: confining runaway excitation by shaping synaptic transmission. *Nat Rev Neurosci.* (2007) 8:935–47. doi: 10.1038/nrn2274
32. Araque A, Carmignoto G, Haydon PG, Oliet SH, Robitaille R, Volterra A. Gliotransmitters travel in time and space. *Neuron* (2014) 81:728–39. doi: 10.1016/j.neuron.2014.02.007
33. Woo DH, Han KS, Shim JW, Yoon BE, Kim E, Bae JY, et al. TREK-1 and Best1 channels mediate fast and slow glutamate release in astrocytes upon GPCR activation. *Cell* (2012) 151:25–40. doi: 10.1016/j.cell.2012.09.005
34. Verleysdonk S, Martin H, Willker W, Leibfritz D, Hamprecht B. Rapid uptake and degradation of glycine by astroglial cells in culture: synthesis and release of serine and lactate. *Glia* (1999) 27:239–48.
35. Saransaari P, Oja SS. Characteristics of hippocampal glycine release in cell-damaging conditions in the adult and developing mouse. *Neurochem Res.* (2001) 26:845–52. doi: 10.1023/A:1011624421505
36. Shibasaki K, Hosoi N, Kaneko R, Tominaga M, Yamada K. Glycine release from astrocytes via functional reversal of GlyT1. *J Neurochem.* (2017) 140:395–403. doi: 10.1111/jnc.13741
37. Pan HC, Chou YC, Sun SH. P2X7 R-mediated Ca(2+) -independent d-serine release via pannexin-1 of the P2X7 R-pannexin-1 complex in astrocytes. *Glia* (2015) 63:877–93. doi: 10.1002/glia.22790
38. Wolosker H, Balu DT, Coyle JT. The rise and fall of the d-serine-mediated gliotransmission hypothesis. *Trends Neurosci.* (2016) 39:712–21. doi: 10.1016/j.tins.2016.09.007
39. Zhu H, Wang N, Yao L, Chen Q, Zhang R, Qian J, et al. Moderate UV exposure enhances learning and memory by promoting a novel glutamate biosynthetic pathway in the brain. *Cell* (2018) 173:1716–27e17. doi: 10.1016/j.cell.2018.04.014
40. Uwechue NM, Marx MC, Chevy Q, Billups B. Activation of glutamate transport evokes rapid glutamine release from perisynaptic astrocytes. *J Physiol.* (2012) 590:2317–31. doi: 10.1113/jphysiol.2011.226605
41. Furuta A, Rothstein JD, Martin LJ. Glutamate transporter protein subtypes are expressed differentially during rat CNS development. *J Neurosci.* (1997) 17:8363–75. doi: 10.1523/JNEUROSCI.17-21-08363.1997
42. Haugeto O, Ullensvang K, Levy LM, Chaudhry FA, Honore T, Nielsen M, et al. Brain glutamate transporter proteins form homomultimers. *J Biol Chem.* (1996) 271:27715–22. doi: 10.1074/jbc.271.44.27715
43. Hu W, MacDonald ML, Elswick DE, Sweet RA. The glutamate hypothesis of schizophrenia: evidence from human brain tissue studies. *Ann N Y Acad Sci.* (2015) 1338:38–57. doi: 10.1111/nyas.12547
44. McCullumsmith RE, O'Donovan SM, Drummond JB, Benesh FS, Simmons M, Roberts R, et al. Cell-specific abnormalities of glutamate transporters in schizophrenia: sick astrocytes and compensating relay neurons? *Mol Psychiatry* (2016) 21:823–30. doi: 10.1038/mp.2015.148
45. O'Donovan SM, Hasselfeld K, Bauer D, Simmons M, Roussos P, Haroutunian V, et al. Glutamate transporter splice variant expression in an enriched pyramidal cell population in schizophrenia. *Transl Psychiatry* (2015) 5:e579. doi: 10.1038/tp.2015.74
46. Schmitt A, Asan E, Lesch KP, Kugler P. A splice variant of glutamate transporter GLT1/EAAT2 expressed in neurons: cloning and localization in rat nervous system. *Neuroscience* (2002) 109:45–61. doi: 10.1016/S0306-4522(01)00451-1
47. Gonzalez-Gonzalez IM, Garcia-Tardon N, Cubelos B, Gimenez C, Zafra F. The glutamate transporter GLT1b interacts with the scaffold protein PSD-95. *J Neurochem.* (2008) 105:1834–48. doi: 10.1111/j.1471-4159.2008.05281.x
48. Underhill SM, Wheeler DS, Amara SG. Differential regulation of two isoforms of the glial glutamate transporter EAAT2 by DLG1 and CaMKII. *J Neurosci.* (2015) 35:5260–70. doi: 10.1523/JNEUROSCI.4365-14.2015
49. Karlsson RM, Tanaka K, Heilig M, Holmes A. Loss of glial glutamate and aspartate transporter (excitatory amino acid transporter 1) causes locomotor hyperactivity and exaggerated responses to psychotomimetics: rescue by haloperidol and metabotropic glutamate 2/3 agonist. *Biol Psychiatry* (2008) 64:810–4. doi: 10.1016/j.biopsych.2008.05.001
50. Karlsson RM, Tanaka K, Saksida LM, Bussey TJ, Heilig M, Holmes A. Assessment of glutamate transporter GLAST (EAAT1)-deficient mice for phenotypes relevant to the negative and executive/cognitive symptoms of schizophrenia. *Neuropsychopharmacology* (2009) 34:1578–89. doi: 10.1038/npp.2008.215
51. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, et al. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science* (1997) 276:1699–702. doi: 10.1126/science.276.5319.1699
52. Kiryk A, Aida T, Tanaka K, Banerjee P, Wilczynski GM, Meyza K, et al. Behavioral characterization of GLT1 (+/-) mice as a model of mild glutamatergic hyperfunction. *Neurotox Res.* (2008) 13:19–30. doi: 10.1007/BF03033364
53. Matsugami TR, Tanemura K, Mieda M, Nakatomi R, Yamada K, Kondo T, et al. From the cover: indispensability of the glutamate transporters GLAST and GLT1 to brain development. *Proc Natl Acad Sci USA.* (2006) 103:12161–6. doi: 10.1073/pnas.0509144103
54. Aida T, Yoshida J, Nomura M, Tanimura A, Iino Y, Soma M, et al. Astroglial glutamate transporter deficiency increases synaptic excitability and leads to pathological repetitive behaviors in mice. *Neuropsychopharmacology* (2015) 40:1569–79. doi: 10.1038/npp.2015.26
55. Arnth-Jensen N, Jabaudon D, Scanziani M. Cooperation between independent hippocampal synapses is controlled by glutamate uptake. *Nat Neurosci.* (2002) 5:325–31. doi: 10.1038/nn825
56. Fairman WA, Vandenberg RJ, Arriza JL, Kavanaugh MP, Amara SG. An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature* (1995) 375:599–603. doi: 10.1038/375599a0
57. Wadiche JI, Amara SG, Kavanaugh MP. Ion fluxes associated with excitatory amino acid transport. *Neuron* (1995) 15:721–8. doi: 10.1016/0896-6273(95)90159-0
58. Veruki ML, Morkve SH, Hartveit E. Activation of a presynaptic glutamate transporter regulates synaptic transmission through electrical signaling. *Nat Neurosci.* (2006) 9:1388–96. doi: 10.1038/nn1793
59. Niklaus S, Cadetti L, Vom Berg-Maurer CM, Lehnherr A, Hotz AL, Forster IC, et al. Shaping of signal transmission at the photoreceptor synapse by EAAT2 glutamate transporters. *eNeuro* (2017) 4:ENEURO.0339-16.2017. doi: 10.1523/ENEURO.0339-16.2017
60. Jelen LA, King S, Mullins PG, Stone JM. Beyond static measures: a review of functional magnetic resonance spectroscopy and its potential to investigate dynamic glutamatergic abnormalities in schizophrenia. *J Psychopharmacol.* (2018) 32:497–508. doi: 10.1177/0269881117747579
61. Merritt K, Egerton A, Kempton MJ, Taylor MJ, McGuire PK. Nature of glutamate alterations in schizophrenia: a meta-analysis of proton magnetic resonance spectroscopy studies. *JAMA Psychiatry* (2016) 73:665–74. doi: 10.1001/jamapsychiatry.2016.0442
62. Kraguljac NV, Frolich MA, Tran S, White DM, Nichols N, Barton-McArdle A, et al. Ketamine modulates hippocampal neurochemistry and functional connectivity: a combined magnetic resonance spectroscopy and resting-state fMRI study in healthy volunteers. *Mol Psychiatry* (2017) 22:562–9. doi: 10.1038/mp.2016.122

63. Hashimoto K, Engberg G, Shimizu E, Nordin C, Lindstrom LH, Iyo M. Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients. *BMC Psychiatry* (2005) 5:6. doi: 10.1186/1471-244X-5-6
64. Bustillo JR, Chen H, Jones T, Lemke N, Abbott C, Qualls C, et al. Increased glutamine in patients undergoing long-term treatment for schizophrenia: a proton magnetic resonance spectroscopy study at 3 T. *JAMA Psychiatry* (2014) 71:265–72. doi: 10.1001/jamapsychiatry.2013.3939
65. Dempster K, Norman R, Theberge J, Densmore M, Schaefer B, Williamson P. Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. *Psychiatry Res.* (2015) 233:180–5. doi: 10.1016/j.pscychresns.2015.06.003
66. Cavus I, Kasoff WS, Cassaday MP, Jacob R, Gueorguieva R, Sherwin RS, et al. Extracellular metabolites in the cortex and hippocampus of epileptic patients. *Ann Neurol.* (2005) 57:226–35. doi: 10.1002/ana.20380
67. Eid T, Thomas MJ, Spencer DD, Runden-Pran E, Lai JC, Malthankar GV, et al. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet* (2004) 363:28–37. doi: 10.1016/S0140-6736(03)15166-5
68. Liang SL, Carlson GC, Coulter DA. Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. *J Neurosci.* (2006) 26:8537–48. doi: 10.1523/JNEUROSCI.0329-06.2006
69. Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, et al. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci.* (2010) 13:584–91. doi: 10.1038/nn.2535
70. Eid T, Ghosh A, Wang Y, Beckstrom H, Zaveri HP, Lee TS, et al. Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. *Brain* (2008) 131(Pt 8):2061–70. doi: 10.1093/brain/awn133
71. Behrens MM, Ali SS, Dao DN, Lucero J, Shekhtman G, Quick KL, et al. Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. *Science* (2007) 318:1645–7. doi: 10.1126/science.1148045
72. Lewis DA, Curley AA, Glausier JR, Volk DW. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. *Trends Neurosci.* (2012) 35:57–67. doi: 10.1016/j.tins.2011.10.004
73. Steullet P, Cabungcal JH, Coyle J, Didriksen M, Gill K, Grace AA, et al. Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. *Mol Psychiatry* (2017) 22:936–43. doi: 10.1038/mp.2017.47
74. Lodge DJ, Behrens MM, Grace AA. A loss of parvalbumin-containing interneurons is associated with diminished oscillatory activity in an animal model of schizophrenia. *J Neurosci.* (2009) 29:2344–54. doi: 10.1523/JNEUROSCI.5419-08.2009
75. Tomasella E, Bechelli L, Ogando MB, Mininni C, Di Guilmi MN, De Fino F, et al. Deletion of dopamine D2 receptors from parvalbumin interneurons in mouse causes schizophrenia-like phenotypes. *Proc Natl Acad Sci USA.* (2018) 115:3476–81. doi: 10.1073/pnas.1719897115
76. Del Pino I, Garcia-Frigola C, Dehorter N, Brotons-Mas JR, Alvarez-Salvado E, Martinez de Lagran M, et al. Erbb4 deletion from fast-spiking interneurons causes schizophrenia-like phenotypes. *Neuron* (2013) 79:1152–68. doi: 10.1016/j.neuron.2013.07.010
77. Kondziella D, Brenner E, Eyjolfsson EM, Markinhuhta KR, Carlsson ML, Sonnewald U. Glial-neuronal interactions are impaired in the schizophrenia model of repeated MK801 exposure. *Neuropsychopharmacology* (2006) 31:1880–7. doi: 10.1038/sj.npp.1300993
78. Labrie V, Wong AH, Roder JC. Contributions of the D-serine pathway to schizophrenia. *Neuropharmacology* (2012) 62:1484–503. doi: 10.1016/j.neuropharm.2011.01.030
79. Hashimoto K, Fukushima T, Shimizu E, Komatsu N, Watanabe H, Shinoda N, et al. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. *Arch Gen Psychiatry* (2003) 60:572–6. doi: 10.1001/archpsyc.60.6.572
80. Bendikov I, Nadri C, Amar S, Panizzutti R, De Miranda J, Wolosker H, et al. A CSF and postmortem brain study of D-serine metabolic parameters in schizophrenia. *Schizophr Res.* (2007) 90:41–51. doi: 10.1016/j.schres.2006.10.010
81. Morita Y, Ujike H, Tanaka Y, Otani K, Kishimoto M, Morio A, et al. A genetic variant of the serine racemase gene is associated with schizophrenia. *Biol Psychiatry* (2007) 61:1200–3. doi: 10.1016/j.biopsych.2006.07.025
82. Labrie V, Roder JC. The involvement of the NMDA receptor D-serine/glycine site in the pathophysiology and treatment of schizophrenia. *Neurosci Biobehav Rev.* (2010) 34:351–72. doi: 10.1016/j.neubiorev.2009.08.002
83. Lane HY, Lin CH, Green MF, Hellemann G, Huang CC, Chen PW, et al. Add-on treatment of benzoate for schizophrenia: a randomized, double-blind, placebo-controlled trial of D-amino acid oxidase inhibitor. *JAMA Psychiatry* (2013) 70:1267–75. doi: 10.1001/jamapsychiatry.2013.2159
84. Lin CH, Lin CH, Chang YC, Huang YJ, Chen PW, Yang HT, et al. Sodium benzoate, a D-amino acid oxidase inhibitor, added to clozapine for the treatment of schizophrenia: a randomized, double-blind, placebo-controlled trial. *Biol Psychiatry* (2017) 84:422–32. doi: 10.1016/j.biopsych.2017.12.006
85. St Clair D, Blackwood D, Muir W, Carothers A, Walker M, Spowart G, et al. Association within a family of a balanced autosomal translocation with major mental illness. *Lancet* (1990) 336:13–6. doi: 10.1016/0140-6736(90)91520-K
86. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, et al. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet.* (2000) 9:1415–23. doi: 10.1093/hmg/9.9.1415
87. Bernstein HG, Dobrowolny H, Keilhoff G, Bogerts B, Steiner J. Reduced density of DISC1 expressing astrocytes in the dentate gyrus but not in the subventricular zone in schizophrenia. *Neuropsychopharmacology* (2018) 43:457–8. doi: 10.1038/npp.2017.242
88. Ma TM, Abazyan S, Abazyan B, Nomura J, Yang C, Seshadri S, et al. Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. *Mol Psychiatry* (2013) 18:557–67. doi: 10.1038/mp.2012.97
89. Xia M, Zhu S, Shevelkin A, Ross CA, Pletnikov M. DISC1, astrocytes and neuronal maturation: a possible mechanistic link with implications for mental disorders. *J Neurochem.* (2016) 138:518–24. doi: 10.1111/jnc.13663
90. Terrillion CE, Abazyan B, Yang Z, Crawford J, Shevelkin AV, Jouroukhin Y, et al. DISC1 in astrocytes influences adult neurogenesis and hippocampus-dependent behaviors in mice. *Neuropsychopharmacology* (2017) 42:2242–51. doi: 10.1038/npp.2017.129
91. Young JW, Geyer MA. Evaluating the role of the alpha-7 nicotinic acetylcholine receptor in the pathophysiology and treatment of schizophrenia. *Biochem Pharmacol.* (2013) 86:1122–32. doi: 10.1016/j.bcp.2013.06.031
92. Duffy AM, Fitzgerald ML, Chan J, Robinson DC, Milner TA, Mackie K, et al. Acetylcholine alpha7 nicotinic and dopamine D2 receptors are targeted to many of the same postsynaptic dendrites and astrocytes in the rodent prefrontal cortex. *Synapse* (2011) 65:1350–67. doi: 10.1002/syn.20977
93. Gahring LC, Persiyanov K, Dunn D, Weiss R, Meyer EL, Rogers SW. Mouse strain-specific nicotinic acetylcholine receptor expression by inhibitory interneurons and astrocytes in the dorsal hippocampus. *J Comp Neurol.* (2004) 468:334–46. doi: 10.1002/cne.10943
94. Shen JX, Yakel JL. Functional alpha7 nicotinic ACh receptors on astrocytes in rat hippocampal CA1 slices. *J Mol Neurosci.* (2012) 48:14–21. doi: 10.1007/s12031-012-9719-3
95. Sharma G, Vijayaraghavan S. Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores. *Proc Natl Acad Sci USA.* (2001) 98:4148–53. doi: 10.1073/pnas.071540198
96. Lin H, Hsu FC, Baumann BH, Coulter DA, Lynch DR. Cortical synaptic NMDA receptor deficits in alpha7 nicotinic acetylcholine receptor gene deletion models: implications for neuropsychiatric diseases. *Neurobiol Dis.* (2014) 63:129–40. doi: 10.1016/j.nbd.2013.11.021
97. Singh NS, Paul RK, Ramamoorthy A, Torjman MC, Moaddel R, Bernier M, et al. Nicotinic acetylcholine receptor antagonists alter the function and expression of serine racemase in PC-12 and 1321N1 cells. *Cell Signal.* (2013) 25:2634–45. doi: 10.1016/j.cellsig.2013.08.025
98. Papouin T, Dunphy JM, Tolman M, Dineley KT, Haydon PG. Septal cholinergic neuromodulation tunes the astrocyte-dependent gating of

hippocampal NMDA receptors to wakefulness. *Neuron* (2017) 94:840–54.e7. doi: 10.1016/j.neuron.2017.04.021

99. Gozzi A, Large CH, Schwarz A, Bertani S, Crestan V, Bifone A. Differential effects of antipsychotic and glutamatergic agents on the phMRI response to phencyclidine. *Neuropsychopharmacology* (2008) 33:1690–703. doi: 10.1038/sj.npp.1301547
100. Poels EM, Kegeles LS, Kantrowitz JT, Javitt DC, Lieberman JA, Abi-Dargham A, et al. Glutamatergic abnormalities in schizophrenia: a review of proton MRS findings. *Schizophr Res.* (2014) 152:325–32. doi: 10.1016/j.schres.2013.12.013
101. Ragland JD, Ranganath C, Harms MP, Barch DM, Gold JM, Layher E, et al. Functional and neuroanatomic specificity of episodic memory dysfunction in schizophrenia: a functional magnetic resonance imaging study of the relational and item-specific encoding task. *JAMA Psychiatry* (2015) 72:909–16. doi: 10.1001/jamapsychiatry.2015.0276
102. Ragland JD, Ranganath C, Barch DM, Gold JM, Haley B, MacDonald AW III, et al. Relational and Item-Specific Encoding (RISE): task development and psychometric characteristics. *Schizophr Bull.* (2012) 38:114–24. doi: 10.1093/schbul/sbr146
103. Ragland JD, Blumenfeld RS, Ramsay IS, Yonelinas A, Yoon J, Solomon M, et al. Neural correlates of relational and item-specific encoding during working and long-term memory in schizophrenia. *Neuroimage* (2012) 59:1719–26. doi: 10.1016/j.neuroimage.2011.08.055
104. Finnema SJ, Scheinin M, Shahid M, Lehto J, Borroni E, Bang-Andersen B, et al. Application of cross-species PET imaging to assess neurotransmitter release in brain. *Psychopharmacology* (2015) 232:4129–57. doi: 10.1007/s00213-015-3938-6
105. Takano H. Cognitive function and monoamine neurotransmission in schizophrenia: evidence from positron emission tomography studies. *Front Psychiatry* (2018) 9:228. doi: 10.3389/fpsyt.2018.00228
106. Vasic N, Connemann BJ, Wolf RC, Tumani H, Brettschneider J. Cerebrospinal fluid biomarker candidates of schizophrenia: where do we stand? *Eur Arch Psychiatry Clin Neurosci.* (2012) 262:375–91. doi: 10.1007/s00406-011-0280-9
107. Lin CH, Chang HT, Chen YJ, Lin CH, Huang CH, Tun R, et al. Distinctively higher plasma G72 protein levels in patients with schizophrenia than in healthy individuals. *Mol Psychiatry* (2014) 19:636–7. doi: 10.1038/mp.2013.80
108. Trepanier MO, Hopperton KE, Mizrahi R, Mechawar N, Bazinet RP. Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. *Mol Psychiatry* (2016) 21:1009–26. doi: 10.1038/mp.2016.90
109. Javitt DC, Carter CS, Krystal JH, Kantrowitz JT, Girgis RR, Kegeles LS, et al. Utility of imaging-based biomarkers for glutamate-targeted drug development in psychotic disorders: a randomized clinical trial. *JAMA Psychiatry* (2018) 75:11–9. doi: 10.1001/jamapsychiatry.2017.3572
110. Girgis RR, Zoghbi AW, Javitt DC, Lieberman JA. The past and future of novel, non-dopamine-2 receptor therapeutics for schizophrenia: a critical and comprehensive review. *J Psychiatr Res.* (2018). doi: 10.1016/j.jpsychires.2018.07.006. [Epub ahead of print].
111. Kantrowitz JT, Woods SW, Petkova E, Cornblatt B, Corcoran CM, Chen H, et al. D-serine for the treatment of negative symptoms in individuals at clinical high risk of schizophrenia: a pilot, double-blind, placebo-controlled, randomised parallel group mechanistic proof-of-concept trial. *Lancet Psychiatry* (2015) 2:403–12. doi: 10.1016/S2215-0366(15)00098-X
112. Harvey RJ, Yee BK. Glycine transporters as novel therapeutic targets in schizophrenia, alcohol dependence and pain. *Nat Rev Drug Discov.* (2013) 12:866–85. doi: 10.1038/nrd3893
113. Fell MJ, McKinzie DL, Monn JA, Svensson KA. Group II metabotropic glutamate receptor agonists and positive allosteric modulators as novel treatments for schizophrenia. *Neuropharmacology* (2012) 62:1473–83. doi: 10.1016/j.neuropharm.2011.06.007

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 06 November 2018
doi: 10.3389/fpsyt.2018.00559

The Emerging Role of Altered D-Aspartate Metabolism in Schizophrenia: New Insights From Preclinical Models and Human Studies

Francesco Errico[1]*, *Tommaso Nuzzo*[2], *Massimo Carella*[2], *Alessandro Bertolino*[3] and *Alessandro Usiello*[4,5]*

[1] Department of Agricultural Sciences, University of Naples "Federico II", Portici, Italy, [2] Translational Neuroscience Unit, IRCCS Casa Sollievo Della Sofferenza, San Giovanni Rotondo, Italy, [3] Group of Psychiatric Neuroscience, Department of Basic Medical Science, Neuroscience and Sense Organs, Aldo Moro University, Bari, Italy, [4] Laboratory of Behavioural Neuroscience, Ceinge Biotecnologie Avanzate, Naples, Italy, [5] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Università Degli Studi Della Campania "Luigi Vanvitelli", Caserta, Italy

OPEN ACCESS

Edited by:
Hsien-Yuan Lane,
China Medical University, Taiwan

Reviewed by:
Chih-Min Liu,
National Taiwan University Hospital, Taiwan
Marek Schwendt,
University of Florida, United States
Maarten Van Den Buuse,
La Trobe University, Australia

***Correspondence:**
Francesco Errico
francesco.errico@unina.it
Alessandro Usiello
usiello@ceinge.unina.it

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: 20 July 2018
Accepted: 17 October 2018
Published: 06 November 2018

Citation:
Errico F, Nuzzo T, Carella M, Bertolino A and Usiello A (2018) The Emerging Role of Altered D-Aspartate Metabolism in Schizophrenia: New Insights From Preclinical Models and Human Studies. Front. Psychiatry 9:559. doi: 10.3389/fpsyt.2018.00559

Besides D-serine, another D-amino acid with endogenous occurrence in the mammalian brain, D-aspartate, has been recently shown to influence NMDA receptor (NMDAR)-mediated transmission. D-aspartate is present in the brain at extracellular level in nanomolar concentrations, binds to the agonist site of NMDARs and activates this subclass of glutamate receptors. Along with its direct effect on NMDARs, D-aspartate can also evoke considerable L-glutamate release in specific brain areas through the presynaptic activation of NMDA, AMPA/kainate and mGlu5 receptors. D-aspartate is enriched in the embryonic brain of rodents and humans and its concentration strongly decreases after birth, due to the post-natal expression of the catabolising enzyme D-aspartate oxidase (DDO). Based on the hypothesis of NMDAR hypofunction in schizophrenia pathogenesis, recent preclinical and clinical studies suggested a relationship between perturbation of D-aspartate metabolism and this psychiatric disorder. Consistently, neurophysiological and behavioral characterization of *Ddo* knockout ($Ddo^{-/-}$) and D-aspartate-treated mice highlighted that abnormally higher endogenous D-aspartate levels significantly increase NMDAR-mediated synaptic plasticity, neuronal spine density and memory. Remarkably, increased D-aspartate levels influence schizophrenia-like phenotypes in rodents, as indicated by improved fronto-hippocampal connectivity, attenuated prepulse inhibition deficits and reduced activation of neuronal circuitry induced by phencyclidine exposure. In healthy humans, a genetic polymorphism associated with reduced prefrontal *DDO* gene expression predicts changes in prefrontal phenotypes including greater gray matter volume and enhanced functional activity during working memory. Moreover, neurochemical detections in *post-mortem* brain of schizophrenia-affected patients have shown significantly reduced D-aspartate content in prefrontal regions, associated with increased *DDO* mRNA expression or DDO enzymatic activity. Overall, these findings

suggest a possible involvement of dysregulated embryonic D-aspartate metabolism in schizophrenia pathophysiology and, in turn, highlight the potential use of free D-aspartate supplementation as a new add-on therapy for treating the cognitive symptoms of this mental illness.

Keywords: D-aspartate, D-serine, schizophrenia, NMDA receptor, D-aspartate oxidase, mouse models

INTRODUCTION

Although L-amino acids are mostly used for protein synthesis and metabolic processes in eukaryotes, some free amino acids are present in a substantial amount in D configuration in mammalian tissues, including humans. In particular, free D-aspartate (D-Asp), and D-serine (D-Ser) are enriched in the brain where they emerge in an age- and region-dependent manner (1–5). Since its discovery at the beginning of the 90s (6), D-Ser has been extensively studied in the mammalian brain. Nowadays, it is well known that D-Ser is an endogenous *N*-Methyl D-Aspartate (NMDA) receptor (NMDAR) co-agonist that regulates the activation of glutamatergic excitatory synapses (7–10), thus influencing different NMDAR-dependent functions, such as brain development (11), synaptic transmission and plasticity (12–17), and behaviors (18–20). In this line, alteration of D-Ser metabolism has been linked to pathological conditions associated with NMDAR dysfunction, including schizophrenia (21–23). Unlike D-Ser, the neurobiological role of D-Asp has been most significantly studied only from the last decade, so it is still poorly defined. Nonetheless, we know today that D-Asp is able to directly modulate NMDAR-mediated transmission and functions in rodents, and dysregulation of its metabolism occurs in the brain of schizophrenia patients. In this review, we will summarize the evidence collected in both animal models and humans that have led to hypothesize a role for this atypical amino acid in schizophrenia pathophysiology.

PRESENCE OF FREE D-ASPARTATE AND ITS METABOLIC REGULATION IN THE MAMMALIAN BRAIN

The presence of free D-Asp in the mammalian brain was reported for the first time by Dunlop et al. in the mid-80s (1). This pioneering observation has been followed by many other studies in rodents and humans that overall showed a transient emergence of D-Asp in the brain, characterized by a peak of concentration during developmental stages and a drastic fall after birth (1–4, 24, 25). Among these studies, HPLC detections obtained by Hashimoto et al. revealed that the amount of D-Asp in the human prefrontal cortex (PFC) homogenates even exceeds that of the corresponding L-form at gestational week 14 (3). Consistent with this finding, in the rat brain D-Asp appears at embryonic day 12 (E12) selectively in non-proliferating neuroblasts but not in mitotic cells of the ventrocaudal forebrain, midbrain, and hindbrain (24). In the ventral side of the forebrain, D-Asp emerges in cell bodies of migrating neuroblasts and then shifts to axons once these cells have reached the outer layer of neural epithelium. Between E14 and E20, D-Asp concentration increases and spreads to the whole brain. In particular, in the cerebral cortex, D-Asp is present in both the cell bodies and projections of neuroblasts (24). In another work, Snyder et al. revealed that D-Asp is present in a substantial amount at birth in the forebrain regions, like the cortex and part of the midbrain (25). At post-natal day 2, D-Asp extends to the hindbrain and cerebellum and is enriched in the subventricular zone and the cortical plate of the cerebral cortex, the CA1-CA3 area and the dentate gyrus of the hippocampus, and the external granular layer of the cerebellum. In this last brain region, D-Asp staining is associated with granule cells that have not yet migrated to their definitive location (25). One week after birth, D-Asp decreases and almost disappears in one-month-old brains (26). Notably, in both prenatal and postnatal phases, D-Asp seems to be present exclusively in neurons but not in glial cells (25, 26).

The peculiar spatiotemporal occurrence of D-Asp in the embryonic brain suggests that this atypical molecule is mainly produced through a *de novo* biosynthetic mechanism (**Figure 1**). Accordingly, a time-dependent accumulation of D-Asp was demonstrated in PC12 cells cultured in D-Asp-free medium (27, 28). Further evidence resulted from primary cultures of rat embryonic neurons in which [^{14}C]-D-Asp was produced starting from [^{14}C]-L-Asp precursor (25). A pyridoxal 5'-phosphate (PLP)-dependent glutamate-oxalacetate transaminase 1-like 1 (Got1l1) was identified as the enzyme that converts L-Asp to D-Asp in the adult mouse brain (29). However, subsequent findings have demonstrated that knockout of *Got1l1* gene does not produce any change in cerebral D-Asp levels of mutant mice (30). Interestingly, two studies have shown that serine racemase (SR), the only known enzyme responsible for D-Ser biosynthesis in the mammalian brain (31), could be involved also in D-Asp production, at least in some brain areas (32, 33). Indeed, *Sr* knockout mice display considerably decreased D-Asp levels in the forebrain regions (around 50–60 %) but not in the cerebellum (32, 33). Remarkably, overexpression of SR in rat PC12 cells resulted in intracellular D-Asp increase while genetic ablation of *Sr* in these cells did not produce changes in this D-amino acid levels (33), further supporting that besides SR an additional enzymatic activity for D-Asp biosynthesis is present in mammals.

While D-Asp biosynthetic pathway is still unclear, the enzyme responsible for D-Asp degradation, D-aspartate oxidase (DDO, or DAPOX EC 1.4.3.1), has been well known for a long time (34). DDO is a peroxisomal flavoenzyme (35, 36) that oxidizes D-Asp in presence of H_2O and O_2, producing α-oxaloacetate, H_2O_2, and NH_4^+ (**Figure 1**) (37–39). DDO is inactive toward D-Ser (40) that is degraded by its homologous flavoenzyme, D-amino acid

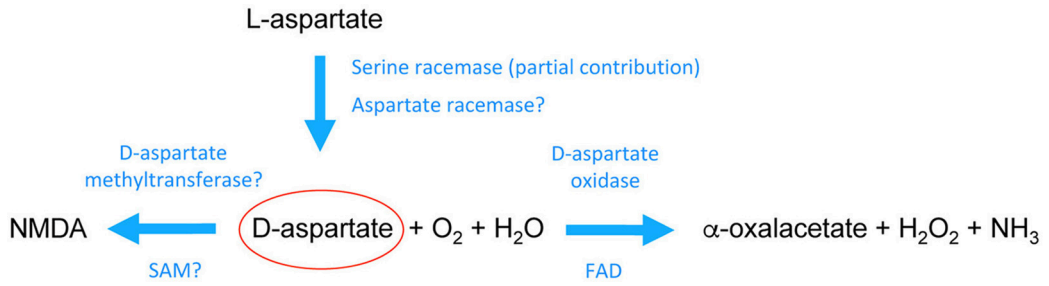

FIGURE 1 | Schematic pathway of the biochemical transformations of D-aspartate in the mammalian brain. D-aspartate is likely generated through stereochemical inversion of L-aspartate. Recent studies in mice have shown that Serine racemase is partially involved in D-aspartate biosynthesis although it is still unclear whether its activity toward aspartate is region- and/or age-dependent. These studies also suggested that there should be a main racemase activity specifically involved in D-aspartate generation. On the other hand, it is acknowledged that D-aspartate is degraded by the enzyme D-aspartate oxidase through a process of deaminative oxidation (requiring flavin adenine dinucleotide, FAD, as a prosthetic group) that produces the ketoacid α-oxalacetate, hydrogen peroxide, and ammonia. In addition, D-aspartate can be converted into its N-methyl derivative, NMDA, possibly through a D-aspartate methyltransferase activity that uses s-adenosylmethionine (SAM) as a methyl donor. See the main text for further descriptions.

oxidase (DAAO, EC 1.4.3.3) (41–43). A specific tripeptide signal at the C-terminus of DDO targets this enzyme to peroxisomes (40, 44), where the toxic H_2O_2 produced by D-Asp oxidation can be safely removed by peroxisome-resident catalases (45).

Consistent with a primary role for DDO activity in controlling its substrate concentration, it has been reported that spatial and temporal expression of this enzyme is reciprocal to D-Asp (26, 35, 46). During mouse lifespan, the postnatal decrease of cerebral D-Asp is mirrored by a time-dependent increase of *Ddo* gene expression, as *Ddo* mRNA levels are very low from E14 to 1 week of age and then strongly rise during the following weeks until adult phase (46). Interestingly, the postnatal increase of *Ddo* transcript levels is temporally associated with concomitant dramatic demethylation of the putative promoter region of *Ddo* gene. In agreement with an influence of DNA methylation on *Ddo* mRNA expression, it has been shown that treatment with the DNA demethylating agent, azacitidine, triggers the expression of *Ddo* transcript in embryonic cortical neurons, which do not express *Ddo* gene in physiological conditions (46). Notably, the temporal pattern of *Ddo* mRNA expression reported in the mouse brain matches closely with the substantial increase in DDO enzymatic activity found in the rat brain during the first weeks of life (35). Like D-Asp, also DDO is prominently localized in neurons, and only marginally expressed in glial population (36). Overall, reciprocal spatiotemporal localization of DDO and D-Asp suggests that during adulthood this enzyme is necessary to remove its endogenous substrate from brain regions where D-Asp is no more required and, indeed, could be functionally detrimental and neurotoxic for neuronal activity and survival, as found in the brain of elderly constitutive knockout mice for *Ddo* gene ($Ddo^{-/-}$) (46–48). The substantial and persistent accumulation of D-Asp in the brain of $Ddo^{-/-}$ mice further suggests that DDO is the only enzyme that catalyzes the endogenous degradation of D-Asp, throughout the entire animal lifespan (47, 49, 50).

PHARMACOLOGICAL FEATURES OF D-ASPARTATE

Pioneering neuropharmacological studies performed in the second half of the 80s, aimed at finding molecules with agonistic or antagonistic activity for L-glutamate (L-Glu) receptors, highlighted that D-Asp is able to bind to the L-Glu site of NMDARs (51–55). In particular, one of these works revealed that the efficiency of D-Asp in displacing the NMDAR antagonist, D-AP5, in rat brain membranes is the same as NMDA and around 10 times lower than L-Glu (54). Many years later, a series of electrophysiological observations demonstrated that D-Asp not only binds to NMDARs but also activates these receptors. Indeed, local application of D-Asp on adult mouse brain slices triggers inward currents in both hippocampal CA1 pyramidal neurons and striatal GABAergic medium spiny neurons, which are strongly reduced by the competitive and non-competitive NMDAR antagonists, D-AP5 and MK-801, respectively (56, 57). D-Asp activates NMDARs through the binding to each of the GluN2 subunits (58), and induces the transient increase of intracellular Ca^{2+} (47). Notably, a small but significant percentage of the currents triggered by D-Asp application still endure even after application of high doses of MK-801 (47, 56, 58). The persistence of D-Asp-mediated currents under conditions of NMDAR blockade suggests that this D-amino acid may be able to affect, at least in part, additional receptor complexes. Accordingly, D-Asp can also stimulate metabotropic mGlu5 receptors in mouse and rat brain (59–61).

The endogenous occurrence of D-Asp in the brain and its main ability to activate NMDARs are consistent with an involvement of this D-amino acid in the *in vivo* modulation of glutamatergic synaptic functioning. In agreement with this idea, early studies have suggested that D-Asp can transit through the extracellular space by transporter mechanisms enabling its release and reuptake. Accordingly, experiments using different *ex vivo* preparations (tissue slices, cells or synaptosomes) demonstrated that D-Asp is released through

exocytotic processes mediated by vesicular Ca^{2+} (25, 62–65). D-Asp is contained in membrane-bound organelles, identified as secretory granules (in PC12 cells) (64) or synaptic vesicles (in synaptosome preparation) (66), in which this D-amino acid is actively stored through still unknown transporter systems. In line with *in vitro* data, Usiello et al. confirmed the existence of Ca^{2+}-dependent D-Asp release in the cortex of living mice. Indeed, in microdialysis studies performed in the PFC of freely moving animals they showed that D-Asp is present in the extracellular space at nanomolar concentration (46, 61) and when dialysates were collected in Ca^{2+}-free artificial cerebrospinal fluid, extracellular concentration of D-Asp dropped below the limit of HPLC detection. Extracellular D-Asp has been also recently demonstrated in the brain of domestic chicks, where it decreases in an age-dependent manner and is transiently induced following high K^+ stimuli (67). The recent evidence that D-Asp occurs also in the extracellular space confirms the idea that this D-amino acid has the potential to bind to NMDARs also *in vivo* and, in turn, to affect the glutamatergic neurotransmission dependent on this receptor subclass. In addition to Ca^{2+}-dependent efflux system, further studies reported that D-Asp may also be released through spontaneous processes (25, 68, 69) or mechanisms involving L-Glu exchange (70, 71).

Different studies suggest that intracellular uptake of D-Asp may depend by L-Glu/L-Asp transporter system, which moves excitatory amino acids against their concentration gradient using the Na^+/K^+ electrochemical gradient. Interestingly, while on one side this carrier system recognizes only the L-enantiomer of Glu, on the other it is able to take up both Asp enantiomers with approximately the same efficiency (72). In line with an involvement of L-Glu/L-Asp transporters in the reuptake of D-Asp, autoradiographic and immunostaining experiments have shown that D-Asp preloading on rodent hippocampal and cerebellar slices causes a strong labeling in glutamatergic axons and in surrounding glial processes (73–75). No direct evidence is so far available about the existence of selective D-Asp reuptake mechanisms in the *in vivo* brain. Nonetheless, the drastic and fast reduction of cortical extracellular D-Asp after experimental removal of Ca^{2+} from the artificial cerebrospinal fluid reported in microdialysis studies (46, 61), seems to suggest the presence of an active and efficient mechanism of D-Asp clearance in the mammalian brain.

PRECLINICAL MODELS WITH A NON-PHYSIOLOGICAL INCREASE OF D-ASPARTATE CONTENT IN THE BRAIN

The generation of *Ddo* knockout mice ($Ddo^{-/-}$) in two independent laboratories (49, 50) has been a turning point for the comprehension of the *in vivo* role of D-Asp and its catabolizing enzyme. Both $Ddo^{-/-}$ mouse lines display higher D-Asp levels in the brain and peripheral organs, but comparable L-Asp (49, 50) and L-Glu content (50), compared to their respective controls. In more detail, HPLC detection in homogenates deriving from the hippocampus, striatum, cortex, cerebellum and olfactory bulbs showed ∼10- to 20-fold increase in D-Asp levels in $Ddo^{-/-}$ mice, compared to wild-type animals (46, 47, 56, 57). Along with D-Asp, also the levels of the N-methyl derivative of D-Asp, NMDA, were increased in $Ddo^{-/-}$ brain homogenates (47, 49). In this regard, biochemical findings indicated that cerebral NMDA could be generated through the transfer of a methyl group from S-adenosyl methionine to D-aspartate catalyzed by a methyltransferase activity (**Figure 1**) (76). In line with the rise of total D-Asp levels in brain homogenates, recent evidence on PFC dialysates has indicated that $Ddo^{-/-}$ mice display significantly increased D-Asp concentration also in the extracellular fraction, compared to $Ddo^{+/+}$ littermates (a rise of about five times, ∼100 nM in $Ddo^{-/-}$ vs. ∼20 nM in $Ddo^{+/+}$) (46).

Besides *Ddo* gene targeting, another experimental approach has been widely used in the last years to raise the cerebral levels of D-Asp. This approach consists of the oral administration of D-Asp to animals for periods of one-two months until the age of two-three months. In relation to the brain region analyzed, such a treatment produces an approximately 2- to 5-fold increase in D-Asp content in homogenate samples, compared to the same areas of untreated mice (56–58, 61). Such rise is substantially lesser than that produced by *Ddo* gene targeting since the DDO enzyme expressed in the brain of adult D-Asp-treated mice is able to limit the further increase of this D-amino acid levels. In line with the effect of *Ddo* gene ablation, also the exogenous administration of D-Asp is able to induce a significant extracellular increase of this molecule in the PFC of freely moving mice, in both chronic and acute conditions (61). In particular, chronic oral administration of D-Asp for 1 month produces an increase in the same order of magnitude as *Ddo* knockout (∼80 nM in treated mice vs. ∼20 nM in untreated mice). On the other hand, acute D-Asp injection (at the dose of 500 mg/kg) is able to trigger a transient and rapid elevation of D-Asp levels already 20 min post-administration (from ∼20 to ∼500 nM). Consistent with the observations of Usiello et al. (61), a very recent study showed that intragastric infusion of D-Asp to rats reaches the hippocampus in 15 min via blood circulation (77). Taken together, these findings have a potential translational relevance as they highlight the ability of D-Asp to cross the blood-brain barrier, as described so far only for the D-stereoisomers of Ser and proline (78, 79). Strikingly, both chronic oral administration and acute injection of D-Asp are able to evoke a significant prefrontal efflux of L-Glu (48, 61) through the presynaptic activation of NMDA, mGlu5, and AMPA/kainate receptors triggered by this D-amino acid (61). Overall, these data indicate that D-Asp could influence glutamatergic neurotransmission not only through its direct binding to the L-Glu site of postsynaptic NMDARs but also through its ability to evoke the presynaptic release of endogenous L-Glu in selective brain regions.

D-ASPARTATE AFFECTS FUNCTIONAL AND STRUCTURAL NEURONAL PROPERTIES DEPENDENT ON NMDA RECEPTORS ACTIVATION

The results described so far suggest that D-Asp stimulates also *in vivo* the glutamatergic transmission in adult rodents. In this

regard, electrophysiological evidence obtained in the last 10 years has revealed that higher D-Asp content enhances NMDAR-dependent early-phase and late-phase long-term potentiation (E-LTP and L-LTP, respectively) in the CA1 area of the hippocampus of both adult *Ddo*$^{-/-}$ and chronically D-Asp-treated mice (47, 56, 58, 80). Interestingly, the induction of E-LTP protocol, which causes a decaying LTP in *Ddo*$^{+/+}$ and untreated mice, is sufficient to maintain an enduring L-LTP (lasting for more than 160 min following tetanic stimulation) in *Ddo*$^{-/-}$ and D-Asp-treated mice (80). In both models, D-Asp-dependent L-LTP is insensitive to rapamycin administration but is suppressed by cytochalasin D (80), a potent inhibitor of actin polymerization. Moreover, chronic treatment with D-Asp increases the frequency of NMDAR-mediated miniature excitatory post-synaptic currents in pyramidal neurons of the medial PFC layer II/III (80). Remarkably, in line with the enhanced glutamatergic transmission, mice chronically treated with D-Asp display stronger metabolic activity in fronto-hippocampal areas, measured by basal cerebral blood volume-weighted functional magnetic resonance imaging (fMRI) (80). In this regard, increased synchronization between the hippocampus and cortical regions has been also recently produced through D-Asp gavage in awake rats subjected to blood oxygen level dependent (BOLD) fMRI (77). Changes in synaptic functioning are commonly associated with structural synaptic variations in dendritic morphology (81, 82). This relationship is observed also in the brain of *Ddo*$^{-/-}$ and D-Asp-treated mice, in which facilitated induction of late-phase synaptic plasticity is mirrored by increased dendritic length and spine density, and greater dendritic arborisation in pyramidal neurons of the PFC and hippocampus (80). These *in vivo* results are inferred also from very recent *in vitro* findings showing that the exogenous application of D-Asp to rat hippocampal slices produces a rapid genesis of middle size spines of CA1 neurons via an actin-sensitive mechanism, as early as 2 h after treatment (77). Consistent with structural and functional enhancement in cortico-hippocampal synaptic plasticity, *Ddo*$^{-/-}$ and D-Asp treated mice display significant improvements in the cognitive domain of spatial memory when tested in the hidden-platform version of the Morris water maze and contextual fear conditioning (47, 57), two behavioral tasks involving the hippocampal activation of NMDARs (83, 84). Similar behavioral observations were reported also in D-Asp-treated rats (85).

However, if on the one hand NMDARs promote synaptic strength and connectivity, on the other they can produce neuronal death if their stimulation is abnormally intense or temporarily too long (86). In line with detrimental NMDAR-related effects, persistent increase of D-Asp levels results in precocious decay of basal glutamatergic transmission, synaptic plasticity and hippocampal reference memory in 13/14-month-old *Ddo*$^{-/-}$ (47), mirrored by loss of excitatory glutamatergic synapses and reduction of synaptic GluN1 and GluN2B subunits (87). In addition, a recent study also revealed that the lack of DDO leads to severe neuroinflammation processes and cell death in an age-dependent manner (46). In line with the results obtained in elderly *Ddo*$^{-/-}$ animals, also aged C57BL/6J mice treated with D-Asp (at the dose of 20 mM) for 12 months display similar deficits in hippocampal NMDAR-mediated synaptic plasticity despite such prolonged administration results in only two-fold D-Asp increase in this brain region (58). However, it should be noted that the interruption of D-Asp treatment for 3 weeks is sufficient to fully rescue the NMDAR-dependent LTP deficits and that such a long administration schedule does not produce locomotor and anxiety-like alterations in aged animals that, on the contrary, display a significant cognitive improvement in cue-dependent fear conditioning paradigm (58). Overall, these data point out that the "Yin and Yang" behavior of NMDARs (86) can be recapitulated in different stages of life by deficient DDO activity in the mouse brain.

D-ASPARTATE AFFECTS SCHIZOPHRENIA-RELATED FEATURES AND ACTIVATION OF NEURONAL CIRCUITS IN THE RODENT BRAIN

Accumulating evidence supports the hypothesis that a developmental hypofunction of NMDARs is a causative factor in the etiology and pathophysiology of schizophrenia (88–91). In the wake of the glutamatergic model of schizophrenia, several clinical, pharmacological, imaging, and genetic findings suggest today that dysfunctional metabolism of the NMDAR co-agonist, D-Ser, may produce NMDAR-mediated alterations leading to the manifestation of this mental illness (21–23, 92).

In the last years, a number of preclinical observations have shown that also D-Asp may contribute to influence some NMDAR-dependent phenotypes related to schizophrenia. For instance, behavioral studies performed in adult *Ddo*$^{-/-}$ and D-Asp-treated mice have revealed that chronic D-Asp elevation significantly reduces the prepulse inhibition (PPI) deficit induced by psychotomimetic drugs like amphetamine and MK-801 without affecting basal properties of sensorimotor filtering (57). In addition, *Ddo*$^{-/-}$ mice display a substantial reduction of motor hyperactivity, ataxia and PPI disruption triggered by acute administration of phencyclidine (PCP) (93, 94), the drug that better than any other models schizophrenia symptoms in both humans and rodents (22, 95–97). In line with behavioral observations, increased levels of D-Asp in *Ddo*$^{-/-}$ mice are also able to counteract the dysfunctional cortico-limbo-thalamic activation induced by PCP, as measured by fMRI (94). Moreover, consistent with increased dendritic length and spine density, resting-state fMRI has shown greater cortico-hippocampal connectivity in the brain of *Ddo*$^{-/-}$ mice (94). Increased functional connectivity between the hippocampus and cortex has been recently found also in the rat brain following intragastric administration of D-Asp (77). Since several clinical and preclinical studies suggest the occurrence of cortico-hippocampal dysconnectivity in schizophrenia (98, 99), the *in vivo* imaging data obtained in animal models with higher D-Asp levels highlight a potential translational relevance for D-Asp in treating this mental illness. Consistently, increased levels of D-Asp are also able to prevent corticostriatal long-term depression (57), a synaptic feature reported also in mice chronically treated with the typical antipsychotic haloperidol (100).

REDUCED D-ASPARTATE CONCENTRATION IN THE HUMAN PREFRONTAL CORTEX OF SCHIZOPHRENIA *POST-MORTEM* BRAIN

Based on the results obtained in preclinical research, recent studies have focused the attention on humans in order to assess D-Asp metabolism and its impact on phenotypes relevant to schizophrenia. So far, two different studies have measured D-Asp levels in two different cohorts of *post-mortem* brain samples deriving from patients with schizophrenia and corresponding non-psychiatric controls (101, 102). The first study, performed on a small number of samples (7–10 subjects/diagnosis), revealed reduced D-Asp levels (about 40%) in the *post-mortem* PFC of schizophrenia-affected patients (101), linked to significantly increased *DDO* mRNA levels in the same brain area (94). However, neither methylation changes in the putative *DDO* promoter nor gross aberrations in this gene, including insertion, deletion, frameshift, or nonsense mutations were found in the same samples (94). Also the second study, performed on a larger number of samples (20 subjects/diagnosis/brain region), reported a significant D-Asp reduction (about 30%) selectively in the dorsolateral PFC (DLPFC) but not in the hippocampus of patients, compared to the respective brain regions of non-psychiatric subjects (102). Interestingly, the biochemical analysis pointed out that reduced content of D-Asp in the DLPFC of schizophrenia-affected subjects is associated with an aberrant increase in DDO enzymatic activity (102). However, differently from the first cohort of samples (101), *DDO* gene expression, as well as DNA methylation status, was comparable between diagnoses (102, 103). Moreover, western blotting analysis of SR, regarded as an enzyme involved in D-Asp biosynthesis (32, 33), revealed no changes between schizophrenia-affected patients and healthy individuals (102).

Furthermore, to assess the effect of putative alteration of D-Asp metabolism in the human brain, another study reported the association among *DDO* gene variations, prefrontal *DDO* mRNA expression and structural/functional prefrontal phenotypes relevant to schizophrenia (80). Within this study, an *in silico* analysis performed on 268 *post-mortem* brains of non-psychiatric subjects (deriving from BrainCloud bank, http://braincloud.jhmi.edu) revealed that the C allele of the single nucleotide polymorphism (SNP), rs3757351, mapping in an intronic region of *DDO* gene, is associated with reduced prefrontal expression of *DDO* transcript, compared to the T allele and, thus, may hypothetically predict increased endogenous D-Asp levels in the PFC. This result led authors to perform *in vivo* imaging analyses whose results disclosed that healthy individuals bearing the C allele also display increased prefrontal gray matter volume and greater prefrontal activity, compared to the subjects with the T allele, when they were subjected to 1- and 2-Back working memory tasks.

Besides the neurochemical and functional studies reported above in *post-mortem* schizophrenia brain, a recent preclinical research by Usiello et al. revealed that the modulation of D-Asp metabolism could be instrumental for the mechanism of action by which a common second-generation antipsychotic, olanzapine, influences glutamatergic cortical neurotransmission (61). In particular, they found that olanzapine, differently from other typical and atypical antipsychotics, inhibits the enzymatic activity of DDO *in vitro*. Moreover, in agreement to *in vitro* results, chronic administration of this antipsychotic is able to increase the extracellular levels of D-Asp and L-Glu in the PFC of freely moving $Ddo^{+/+}$ mice, but not in $Ddo^{-/-}$ animals (61).

CONCLUSIONS AND FUTURE PERSPECTIVES

The findings described in the present review highlight that increased levels of the endogenous NMDAR agonist, D-Asp, impact on a series of functional, and structural phenotypes relevant to schizophrenia. Despite the knowledge on the neurobiological role of D-Asp and its potential involvement in schizophrenia is significantly enhanced in the last years, many issues remain still unsolved or deserve further clarification. For instance, it seems clear today that D-Asp is synthesized in the brain where it is subjected to release and reuptake mechanisms that enable its neuromodulatory activity, but it is still unclear what are the specific enzymes and transport systems responsible for such processes. Is there a selective enzymatic activity responsible for D-Asp production in the mammalian brain? Moreover, the influence of SR upon the endogenous D-aspartate level still waits to be clarified in the human brain. If SR is actually involved in D-Asp biosynthesis in the human brain, to what extent it contributes to the generation of this D-amino acid and in which spatiotemporal window? Are there relevant genetic *SR* variants associated to dysregulation of cerebral D-Asp levels? These questions may help to elucidate the effective significance of altered D-Asp metabolism in schizophrenia since *SR* is in the list of the largest genome-wide association study as a susceptibility gene for schizophrenia (92). The assessment of a D-Asp involvement in schizophrenia will necessarily undergo the verification of altered D-Asp amount in further schizophrenia brains. Indeed, the results of a dysregulated D-Asp metabolism in *post-mortem* brain samples so far achieved are encouraging but refer only to a limited number of samples, analyzed by the same research team (101, 102). Therefore, this issue deserves to be further assessed also by other laboratories and in a larger number of *post-mortem* brain tissues as well as in other biological materials such as the peripheral blood and cerebrospinal fluid of patients (FE, AB and AU personal communication).

Another important aim that should be pursued in future studies concerns the evaluation of the potential therapeutic value of D-Asp in schizophrenia treatment. In support of its translational application, D-Asp is nowadays approved for the use in humans, and commercialized as a dietary supplement. Accordingly, recent studies have shown that D-Asp treatment has no toxicological consequences or influences on hormonal activity of the hypothalamic-pituitary-gonadal axis and the mass of skeletal muscle (104–108). Besides D-Asp administration, future therapeutic strategies may be disclosed by the identification of compounds with inhibitory activity against DDO and, thus, able

to indirectly enhance the availability of cerebral D-Asp. Novel DDO inhibitors have been so far functionally assayed *in vitro* by Homma et al. (109) and await further *in vivo* characterization to test their clinical validity.

Finally, future research needs to provide clear evidence of the potential role of embryonic D-Asp metabolism in the pathophysiological mechanisms leading to schizophrenia. In this regard, it is very important to underline that the etiology of this psychiatric disorder is likely to involve genetic and environmental risk factors emerging during the neurodevelopmental stages (110–113), in coincidence with the peak of D-Asp, and affecting several processes controlled by NMDARs, such as neurogenesis, survival, migration and formation of brain circuits (114–116). Therefore, we speculate that dysfunctional D-Asp metabolism occurring during neurodevelopment may affect early critical processes dependent on NMDARs and, in turn, contribute to schizophrenia vulnerability. The recent generation of a genetic mouse model with prenatal reduction of D-Asp levels (FE and AU personal communication) may aid to disclose the importance of the transient occurrence of D-Asp in developmental brain processes and, in turn, the potential involvement of dysregulated D-Asp metabolism in a neurodevelopmental psychiatric disorder like schizophrenia.

AUTHOR CONTRIBUTIONS

FE and AU conceived and wrote the manuscript. TN, MC and AB critically reviewed the manuscript.

REFERENCES

1. Dunlop DS, Neidle A, McHale D, Dunlop DM, Lajtha A. The presence of free D-aspartic acid in rodents and man. *Biochem Biophys Res Commun.* (1986) 141:27–32. doi: 10.1016/S0006-291X(86)80329-1
2. Neidle A, Dunlop DS. Developmental changes in free D-aspartic acid in the chicken embryo and in the neonatal rat. *Life Sci.* (1990) 46:1517–22. doi: 10.1016/0024-3205(90)90424-P
3. Hashimoto A, Kumashiro S, Nishikawa T, Oka T, Takahashi K, Mito T, et al. Embryonic development and postnatal changes in free D-aspartate and D-serine in the human prefrontal cortex. *J Neurochem.* (1993) 61:348–51. doi: 10.1111/j.1471-4159.1993.tb03575.x
4. Hashimoto A, Oka T, Nishikawa T. Anatomical distribution and postnatal changes in endogenous free D-aspartate and D-serine in rat brain and periphery. *Eur J Neurosci.* (1995) 7:1657–63. doi: 10.1111/j.1460-9568.1995.tb00687.x
5. Hamase K, Homma H, Takigawa Y, Fukushima T, Santa T, Imai K. Regional distribution and postnatal changes of D-amino acids in rat brain. *Biochim Biophys Acta* (1997) 1334:214–22. doi: 10.1016/S0304-4165(96)00095-5
6. Hashimoto A, Nishikawa T, Hayashi T, Fujii N, Harada K, Oka T, et al. The presence of free D-serine in rat brain. *FEBS Lett.* (1992) 296:33–6. doi: 10.1016/0014-5793(92)80397-Y
7. Martineau M, Baux G, Mothet JP. D-serine signalling in the brain: friend and foe. *Trends Neurosci.* (2006) 29:481–91. doi: 10.1016/j.tins.2006.06.008
8. Billard JM. D-Amino acids in brain neurotransmission and synaptic plasticity. *Amino Acids* (2012) 43:1851–60. doi: 10.1007/s00726-012-1346-3
9. Van Horn MR, Sild M, Ruthazer ES. D-serine as a gliotransmitter and its roles in brain development and disease. *Front Cell Neurosci.* (2013) 7:39. doi: 10.3389/fncel.2013.00039
10. Wolosker H, Radzishevsky I. The serine shuttle between glia and neurons: implications for neurotransmission and neurodegeneration. *Biochem Soc Trans.* (2013) 41:1546–50. doi: 10.1042/BST20130220
11. Kim PM, Aizawa H, Kim PS, Huang AS, Wickramasinghe SR, Kashani AH, et al. Serine racemase: activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration. *Proc Natl Acad Sci USA.* (2005) 102:2105–10. doi: 10.1073/pnas.0409723102
12. Mothet JP, Parent AT, Wolosker H, Brady RO Jr, Linden DJ, Ferris CD, et al. D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA.* (2000) 97:4926–31. doi: 10.1073/pnas.97.9.4926
13. Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, et al. Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. *Proc Natl Acad Sci USA.* (2003) 100:15194–9. doi: 10.1073/pnas.2431073100
14. Fossat P, Turpin FR, Sacchi S, Dulong J, Shi T, Rivet JM, et al. Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. *Cereb Cortex* (2012) 22:595–606. doi: 10.1093/cercor/bhr130
15. Papouin T, Ladepeche L, Ruel J, Sacchi S, Labasque M, Hanini M, et al. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. *Cell* (2012) 150:633–46. doi: 10.1016/j.cell.2012.06.029
16. Li Y, Sacchi S, Pollegioni L, Basu AC, Coyle JT, Bolshakov VY. Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. *Nat Commun.* (2013) 4:1760. doi: 10.1038/ncomms2779
17. Rosenberg D, Artoul S, Segal AC, Kolodney G, Radzishevsky I, Dikopoltsev E, et al. Neuronal D-serine and glycine release via the Asc-1 transporter regulates NMDA receptor-dependent synaptic activity. *J Neurosci.* (2013) 33:3533–44. doi: 10.1523/JNEUROSCI.3836-12.2013
18. Basu AC, Tsai GE, Ma CL, Ehmsen JT, Mustafa AK, Han L, et al. Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. *Mol Psychiatry* (2009) 14:719–27. doi: 10.1038/mp.2008.130
19. Labrie V, Duffy S, Wang W, Barger SW, Baker GB, Roder JC. Genetic inactivation of D-amino acid oxidase enhances extinction and reversal learning in mice. *Learn Mem.* (2009) 16:28–37. doi: 10.1101/lm.1112209
20. DeVito LM, Balu DT, Kanter BR, Lykken C, Basu AC, Coyle JT, et al. Serine racemase deletion disrupts memory for order and alters cortical dendritic morphology. *Genes Brain Behav.* (2011) 10:210–22. doi: 10.1111/j.1601-183X.2010.00656.x
21. Coyle JT, Tsai G. The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. *Psychopharmacology* (2004) 174:32–8. doi: 10.1007/s00213-003-1709-2
22. Javitt DC, Zukin SR, Heresco-Levy U, Umbricht D. Has an angel shown the way? Etiological and therapeutic implications of the PCP/NMDA model of schizophrenia *Schizophr Bull.* (2012) 38:958–66. doi: 10.1093/schbul/sbs069
23. Labrie V, Wong AH, Roder JC. Contributions of the D-serine pathway to schizophrenia. *Neuropharmacology* (2012) 62:1484–503. doi: 10.1016/j.neuropharm.2011.01.030
24. Sakai K, Homma H, Lee JA, Fukushima T, Santa T, Tashiro K, et al. Emergence of D-aspartic acid in the differentiating neurons of the rat central nervous system. *Brain Res.* (1998) 808:65–71. doi: 10.1016/S0006-8993(98)00599-X
25. Wolosker H, D'Aniello A, Snyder SH. D-aspartate disposition in neuronal and endocrine tissues: ontogeny, biosynthesis and release. *Neuroscience* (2000) 100:183–9. doi: 10.1016/S0306-4522(00)00321-3
26. Schell MJ, Cooper OB, Snyder SH. D-aspartate localizations imply neuronal and neuroendocrine roles. *Proc Natl Acad Sci USA.* (1997) 94:2013–8. doi: 10.1073/pnas.94.5.2013
27. Long Z, Homma H, Lee JA, Fukushima T, Santa T, Iwatsubo T, et al. Biosynthesis of D-aspartate in mammalian cells. *FEBS Lett.* (1998) 434:231–5. doi: 10.1016/S0014-5793(98)00986-7
28. Long Z, Sekine M, Adachi M, Furuchi T, Imai K, Nimura N, et al. Cell density inversely regulates D- and L-aspartate levels in rat

pheochromocytoma MPT1 cells. *Arch Biochem Biophys.* (2002) 404:92–7. doi: 10.1016/S0003-9861(02)00241-2
29. Kim PM, Duan X, Huang AS, Liu CY, Ming GL, Song H, et al. Aspartate racemase, generating neuronal D-aspartate, regulates adult neurogenesis. *Proc Natl Acad Sci USA.* (2010) 107:3175–9. doi: 10.1073/pnas.0914706107
30. Tanaka-Hayashi A, Hayashi S, Inoue R, Ito T, Konno K, Yoshida T, et al. Is D-aspartate produced by glutamic-oxaloacetic transaminase-1 like 1 (Got1l1): a putative aspartate racemase? *Amino Acids* (2014) 47:79–86. doi: 10.1007/s00726-014-1847-3
31. Wolosker H, Sheth KN, Takahashi M, Mothet JP, Brady RO Jr, Ferris CD, et al. Purification of serine racemase: biosynthesis of the neuromodulator D-serine. *Proc Natl Acad Sci USA.* (1999) 96:721–5. doi: 10.1073/pnas.96.2.721
32. Horio M, Ishima T, Fujita Y, Inoue R, Mori H, Hashimoto K. Decreased levels of free D-aspartic acid in the forebrain of serine racemase (Srr) knock-out mice. *Neurochem Int.* (2013) 62:843–7. doi: 10.1016/j.neuint.2013.02.015
33. Ito T, Hayashida M, Kobayashi S, Muto N, Hayashi A, Yoshimura T, et al. Serine racemase is involved in d-aspartate biosynthesis. *J Biochem.* (2016) 160:345–35. doi: 10.1093/jb/mvw043
34. Still JL, Buell MV, Mary V, knox E, Green DE. Studies on the cyclophorase system; D-aspartic oxidase. *J Biol Chem.* (1949) 179:831–7.
35. Van Veldhoven PP, Brees C, Mannaerts GP. D-aspartate oxidase, a peroxisomal enzyme in liver of rat and man. *Biochim Biophys Acta* (1991) 1073:203–8. doi: 10.1016/0304-4165(91)90203-S
36. Zaar K, Kost HP, Schad A, Volkl A, Baumgart E, Fahimi HD. Cellular and subcellular distribution of D-aspartate oxidase in human and rat brain. *J Comp Neurol.* (2002) 450:272–82. doi: 10.1002/cne.10320
37. D'Aniello A, Vetere A, Petrucelli L. Further study on the specificity of D-amino acid oxidase and D-aspartate oxidase and time course for complete oxidation of D-amino acids. *Comp Biochem Physiol B.* (1993) 105:731–4. doi: 10.1016/0305-0491(93)90113-J
38. Katane M, Homma H. D-aspartate oxidase: the sole catabolic enzyme acting on free D-aspartate in mammals. *Chem Biodivers* (2010) 7:1435–49. doi: 10.1002/cbdv.200900250
39. Katane M, Homma H. D-Aspartate–an important bioactive substance in mammals: a review from an analytical and biological point of view. *J Chromatogr B Analyt Technol Biomed Life Sci.* (2011) 879:3108–21. doi: 10.1016/j.jchromb.2011.03.062
40. Setoyama C, Miura R. Structural and functional characterization of the human brain D-aspartate oxidase. *J Biochem.* (1997) 121:798–803. doi: 10.1093/oxfordjournals.jbchem.a021655
41. Negri A, Ceciliani F, Tedeschi G, Simonic T, Ronchi S. The primary structure of the flavoprotein D-aspartate oxidase from beef kidney. *J Biol Chem.* (1992) 267:11865–71.
42. Pollegioni L, Piubelli L, Sacchi S, Pilone MS, Molla G. Physiological functions of D-amino acid oxidases: from yeast to humans. *Cell Mol Life Sci.* (2007) 64:1373–94. doi: 10.1007/s00018-007-6558-4
43. Sacchi S, Caldinelli L, Cappelletti P, Pollegioni L, Molla G. Structure-function relationships in human D-amino acid oxidase. *Amino Acids* (2012) 43:1833–50. doi: 10.1007/s00726-012-1345-4
44. Amery L, Brees C, Baes M, Setoyama C, Miura R, Mannaerts GP, et al. C-terminal tripeptide Ser-Asn-Leu (SNL) of human D-aspartate oxidase is a functional peroxisome-targeting signal. *Biochem J.* (1998) 336:367–71. doi: 10.1042/bj3360367
45. Beard ME. D-aspartate oxidation by rat and bovine renal peroxisomes: an electron microscopic cytochemical study. *J Histochem Cytochem.* (1990) 38:1377–81.
46. Punzo D, Errico F, Cristino L, Sacchi S, Keller S, Belardo C, et al. Age-Related changes in D-aspartate oxidase promoter methylation control extracellular D-aspartate levels and prevent precocious cell death during brain aging. *J Neurosci.* (2016) 36:3064–78. doi: 10.1523/JNEUROSCI.3881-15.2016
47. Errico F, Nistico R, Napolitano F, Oliva AB, Romano R, Barbieri F, et al. Persistent increase of D-aspartate in D-aspartate oxidase mutant mice induces a precocious hippocampal age-dependent synaptic plasticity and spatial memory decay. *Neurobiol Aging* (2011) 32:2061–74. doi: 10.1016/j.neurobiolaging.2009.12.007
48. Cristino L, Luongo L, Squillace M, Paolone G, Mango D, Piccinin S, et al. D-Aspartate oxidase influences glutamatergic system homeostasis in mammalian brain. *Neurobiol Aging* (2015) 36:1890–902. doi: 10.1016/j.neurobiolaging.2015.02.003
49. Errico F, Pirro MT, Affuso A, Spinelli P, De Felice M, D'Aniello A, et al. A physiological mechanism to regulate D-aspartic acid and NMDA levels in mammals revealed by D-aspartate oxidase deficient mice. *Gene* (2006) 374:50–7. doi: 10.1016/j.gene.2006.01.010
50. Huang AS, Beigneux A, Weil ZM, Kim PM, Molliver ME, Blackshaw S, et al. D-aspartate regulates melanocortin formation and function: behavioral alterations in D-aspartate oxidase-deficient mice. *J Neurosci.* (2006) 26:2814–9. doi: 10.1523/JNEUROSCI.5060-05.2006
51. Fagg GE, Matus A. Selective association of N-methyl aspartate and quisqualate types of L-glutamate receptor with brain postsynaptic densities. *Proc Natl Acad Sci USA.* (1984) 81:6876–80. doi: 10.1073/pnas.81.21.6876
52. Monahan JB, Michel J. Identification and characterization of an N-methyl-D-aspartate-specific L-[3H]glutamate recognition site in synaptic plasma membranes. *J Neurochem.* (1987) 48:1699–708. doi: 10.1111/j.1471-4159.1987.tb05726.x
53. Ogita K, Yoneda Y. Disclosure by triton X-100 of NMDA-sensitive [3H] glutamate binding sites in brain synaptic membranes. *Biochem Biophys Res Commun.* (1988) 153:510–7. doi: 10.1016/S0006-291X(88)81124-0
54. Olverman HJ, Jones AW, Mewett KN, Watkins JC. Structure/activity relations of N-methyl-D-aspartate receptor ligands as studied by their inhibition of [3H]D-2-amino-5-phosphonopentanoic acid binding in rat brain membranes. *Neuroscience* (1988) 26:17–31. doi: 10.1016/0306-4522(88)90124-8
55. Ransom RW, Stec NL. Cooperative modulation of [3H]MK-801 binding to the N-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. *J Neurochem.* (1988) 51:830–6. doi: 10.1111/j.1471-4159.1988.tb01818.x
56. Errico F, Nistico R, Palma G, Federici M, Affuso A, Brilli E, et al. Increased levels of d-aspartate in the hippocampus enhance LTP but do not facilitate cognitive flexibility. *Mol Cell Neurosci.* (2008) 37:236–46. doi: 10.1016/j.mcn.2007.09.012
57. Errico F, Rossi S, Napolitano F, Catuogno V, Topo E, Fisone G, et al. D-aspartate prevents corticostriatal long-term depression and attenuates schizophrenia-like symptoms induced by amphetamine and MK-801. *J Neurosci.* (2008) 28:10404–14. doi: 10.1523/JNEUROSCI.1618-08.2008
58. Errico F, Nistico R, Napolitano F, Mazzola C, Astone D, Pisapia T, et al. Increased D-aspartate brain content rescues hippocampal age-related synaptic plasticity deterioration of mice. *Neurobiol Aging* (2011) 32:2229–43. doi: 10.1016/j.neurobiolaging.2010.01.002
59. Molinaro G, Pietracupa S, Di Menna L, Pescatori L, Usiello A, Battaglia G, et al. D-aspartate activates mGlu receptors coupled to polyphosphoinositide hydrolysis in neonate rat brain slices. *Neurosci Lett.* (2010) 478:128–30. doi: 10.1016/j.neulet.2010.04.077
60. Krashia P, Ledonne A, Nobili A, Cordella A, Errico F, Usiello A, et al. Persistent elevation of D-Aspartate enhances NMDA receptor-mediated responses in mouse substantia nigra pars compacta dopamine neurons. *Neuropharmacology* (2016) 103:69–78. doi: 10.1016/j.neuropharm.2015.12.013
61. Sacchi S, Novellis V, Paolone G, Nuzzo T, Iannotta M, Belardo C, et al. Olanzapine, but not clozapine, increases glutamate release in the prefrontal cortex of freely moving mice by inhibiting D-aspartate oxidase activity. *Sci Rep.* (2017) 7:46288. doi: 10.1038/srep46288
62. Davies LP, Johnston GA. Uptake and release of D- and L-aspartate by rat brain slices. *J Neurochem.* (1976) 26:1007–14. doi: 10.1111/j.1471-4159.1976.tb06485.x
63. Malthe-Sorenssen D, Skrede KK, Fonnum F. Calcium-dependent release of D-[3H]aspartate evoked by selective electrical stimulation of excitatory afferent fibres to hippocampal pyramidal cells *in vitro*. *Neuroscience* (1979) 4:1255–63. doi: 10.1016/0306-4522(79)90155-6
64. Nakatsuka S, Hayashi M, Muroyama A, Otsuka M, Kozaki S, Yamada H, et al. D-Aspartate is stored in secretory granules and released through a Ca(2+)-dependent pathway in a subset of rat pheochromocytoma PC12 cells. *J Biol Chem.* (2001) 276:26589–96. doi: 10.1074/jbc.M011754200

65. D'Aniello S, Somorjai I, Garcia-Fernandez J, Topo E, D'Aniello A. D-Aspartic acid is a novel endogenous neurotransmitter. *FASEB J.* (2010) 25:1014–27. doi: 10.1096/fj.10-168492
66. Fleck MW, Barrionuevo G, Palmer AM. Synaptosomal and vesicular accumulation of L-glutamate, L-aspartate and D-aspartate. *Neurochem Int.* (2001) 39:217–25. doi: 10.1016/S0197-0186(01)00018-3
67. Zachar G, Jako T, Vincze I, Wagner Z, Tabi T, Balint E, et al. Age-related and function-dependent regional alterations of free L- and D-aspartate in postembryonic chick brain. *Acta Biol Hung.* (2018) 69:1–15. doi: 10.1556/018.68.2018.1.1
68. Adachi M, Koyama H, Long Z, Sekine M, Furuchi T, Imai K, et al. L-Glutamate in the extracellular space regulates endogenous D-aspartate homeostasis in rat pheochromocytoma MPT1 cells. *Arch Biochem Biophys.* (2004) 424:89–96. doi: 10.1016/j.abb.2004.01.016
69. Koyama H, Adachi M, Sekine M, Katane M, Furuchi T, Homma H. Cytoplasmic localization and efflux of endogenous D-aspartate in pheochromocytoma 12 cells. *Arch Biochem Biophys.* (2006) 446:131–9. doi: 10.1016/j.abb.2005.12.008
70. Anderson CM, Bridges RJ, Chamberlin AR, Shimamoto K, Yasuda-Kamatani Y, Swanson RA. Differing effects of substrate and non-substrate transport inhibitors on glutamate uptake reversal. *J Neurochem.* (2001) 79:1207–16. doi: 10.1046/j.1471-4159.2001.00668.x
71. Bak LK, Schousboe A, Waagepetersen HS. Characterization of depolarization-coupled release of glutamate from cultured mouse cerebellar granule cells using DL-threo-beta-benzyloxyaspartate (DL-TBOA) to distinguish between the vesicular and cytoplasmic pools. *Neurochem Int.* (2003) 43:417–24. doi: 10.1016/S0197-0186(03)00030-5
72. Palacin M, Estevez R, Bertran J, Zorzano A. Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol Rev.* (1998) 78:969–1054. doi: 10.1152/physrev.1998.78.4.969
73. Taxt T, Storm-Mathisen J. Uptake of D-aspartate and L-glutamate in excitatory axon terminals in hippocampus: autoradiographic and biochemical comparison with gamma-aminobutyrate and other amino acids in normal rats and in rats with lesions. *Neuroscience* (1984) 11:79–100. doi: 10.1016/0306-4522(84)90215-X
74. Garthwaite G, Garthwaite J. Sites of D-[3H]aspartate accumulation in mouse cerebellar slices. *Brain Res.* (1985) 343:129–36. doi: 10.1016/0006-8993(85)91166-7
75. Gundersen V, Danbolt NC, Ottersen OP, Storm-Mathisen J. Demonstration of glutamate/aspartate uptake activity in nerve endings by use of antibodies recognizing exogenous D-aspartate. *Neuroscience* (1993) 57:97–111. doi: 10.1016/0306-4522(93)90114-U
76. D'Aniello G, Tolino A, D'Aniello A, Errico F, Fisher GH, Di Fiore MM. The role of D-aspartic acid and N-methyl-D-aspartic acid in the regulation of prolactin release. *Endocrinology* (2000) 141:3862–70. doi: 10.1210/endo.141.10.7706
77. Kitamura A, Hojo Y, Ikeda M, Karakawa S, Kuwahara T, Kim J, et al. Ingested D-aspartate facilitates the functional connectivity and modifies dendritic spine morphology in rat hippocampus. *Cereb Cortex* (2018). doi: 10.1093/cercor/bhy120. [Epub ahead of print].
78. Bauer D, Hamacher K, Broer S, Pauleit D, Palm C, Zilles K, et al. Preferred stereoselective brain uptake of d-serine–a modulator of glutamatergic neurotransmission. *Nucl Med Biol.* (2005) 32:793–7. doi: 10.1016/j.nucmedbio.2005.07.004
79. Langen KJ, Hamacher K, Bauer D, Broer S, Pauleit D, Herzog H, et al. Preferred stereoselective transport of the D-isomer of cis-4-[18F]fluoro-proline at the blood-brain barrier. *J Cereb Blood Flow Metab.* (2005) 25:607–16. doi: 10.1038/sj.jcbfm.9600065
80. Errico F, Nistico R, Di Giorgio A, Squillace M, Vitucci D, Galbusera A, et al. Free D-aspartate regulates neuronal dendritic morphology, synaptic plasticity, gray matter volume and brain activity in mammals. *Transl Psychiatry* (2014) 4:e417. doi: 10.1038/tp.2014.59
81. Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. Structural basis of long-term potentiation in single dendritic spines. *Nature* (2004) 429:761–6. doi: 10.1038/nature02617
82. Okamoto K, Nagai T, Miyawaki A, Hayashi Y. Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nat Neurosci.* (2004) 7:1104–12. doi: 10.1038/nn1311
83. Morris RG. Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation *in vivo* by the N-methyl-D-aspartate receptor antagonist AP5. *J Neurosci.* (1989) 9:3040–57. doi: 10.1523/JNEUROSCI.09-09-03040.1989
84. Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci.* (1992) 106:274–85. doi: 10.1037/0735-7044.106.2.274
85. Topo E, Soricelli A, Di Maio A, D'Aniello E, Di Fiore MM, D'Aniello A. Evidence for the involvement of D-aspartic acid in learning and memory of rat. *Amino Acids* (2010) 38:1561–9. doi: 10.1007/s00726-009-0369-x
86. Hardingham GE, Bading H. The Yin and Yang of NMDA receptor signalling. *Trends Neurosci.* (2003) 26:81–9. doi: 10.1016/S0166-2236(02)00040-1
87. Cristino L, Luongo L, Squillace M, Paolone G, Mango D, Piccinin S, et al. D-Aspartate-Oxidase influences glutamatergic system homeostasis in mammalian brain. *Neurobiol Aging.* (2015) 36:1890–902. doi: 10.1016/j.neurobiolaging.2015.02.00
88. Sawa A, Snyder SH. Schizophrenia: neural mechanisms for novel therapies. *Mol Med.* (2003) 9:3–9. doi: 10.1007/BF03402101
89. Coyle JT, Tsai G. NMDA receptor function, neuroplasticity, and the pathophysiology of schizophrenia. *Int Rev Neurobiol.* (2004) 59:491–515. doi: 10.1016/S0074-7742(04)59019-0
90. Coyle JT. NMDA receptor and schizophrenia: a brief history. *Schizophr Bull.* (2012) 38:920–6. doi: 10.1093/schbul/sbs076
91. Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* (2012) 37:4–15. doi: 10.1038/npp.2011.181
92. Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* (2014) 511:421–7. doi: 10.1038/nature13595
93. de Bartolomeis A, Errico F, Aceto G, Tomasetti C, Usiello A, Iasevoli F. D-aspartate dysregulation in Ddo(-/-) mice modulates phencyclidine-induced gene expression changes of postsynaptic density molecules in cortex and striatum. *Prog Neuropsychopharmacol Biol Psychiatry* (2015) 62:35–43. doi: 10.1016/j.pnpbp.2015.05.003
94. Errico F, D'Argenio V, Sforazzini F, Iasevoli F, Squillace M, Guerri G, et al. A role for D-aspartate oxidase in schizophrenia and in schizophrenia-related symptoms induced by phencyclidine in mice. *Transl Psychiatry* (2015) 5:e512. doi: 10.1038/tp.2015.2
95. Morris BJ, Cochran SM, Pratt JA. PCP: from pharmacology to modelling schizophrenia. *Curr Opin Pharmacol.* (2005) 5:101–6. doi: 10.1016/j.coph.2004.08.008
96. Jones CA, Watson DJ, Fone KC. Animal models of schizophrenia. *Br J Pharmacol.* (2011) 164:1162–94. doi: 10.1111/j.1476-5381.2011.01386.x
97. Moghaddam B, Krystal JH. Capturing the angel in "angel dust": twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. *Schizophr Bull.* (2012) 38:942–9. doi: 10.1093/schbul/sbs075
98. Zhou Y, Shu N, Liu Y, Song M, Hao Y, Liu H, et al. Altered resting-state functional connectivity and anatomical connectivity of hippocampus in schizophrenia. *Schizophr Res.* (2008) 100:120–32. doi: 10.1016/j.schres.2007.11.039
99. Meyer-Lindenberg A. From maps to mechanisms through neuroimaging of schizophrenia. *Nature* (2010) 468:194–202. doi: 10.1038/nature09569
100. Centonze D, Usiello A, Costa C, Picconi B, Erbs E, Bernardi G, et al. Chronic haloperidol promotes corticostriatal long-term potentiation by targeting dopamine D2L receptors. *J Neurosci.* (2004) 24:8214–22. doi: 10.1523/JNEUROSCI.1274-04.2004
101. Errico F, Napolitano F, Squillace M, Vitucci D, Blasi G, de Bartolomeis A, et al. Decreased levels of d-aspartate and NMDA in the prefrontal cortex and striatum of patients with schizophrenia. *J Psychiatr Res.* (2013) 47:1432–7. doi: 10.1016/j.jpsychires.2013.06.013
102. Nuzzo T, Sacchi S, Errico F, Keller S, Palumbo O, Florio E, et al. Decreased free d-aspartate levels are linked to enhanced d-aspartate oxidase activity in the dorsolateral prefrontal cortex of schizophrenia patients. *NPJ Schizophr.* (2017) 3:16. doi: 10.1038/s41537-017-0015-7

103. Keller S, Punzo D, Cuomo M, Affinito O, Coretti L, Sacchi S, et al. DNA methylation landscape of the genes regulating D-serine and D-aspartate metabolism in post-mortem brain from controls and subjects with schizophrenia. *Sci Rep.* (2018) 8:10163. doi: 10.1038/s41598-018-28332-x
104. Willoughby DS, Leutholtz B. D-aspartic acid supplementation combined with 28 days of heavy resistance training has no effect on body composition, muscle strength, and serum hormones associated with the hypothalamo-pituitary-gonadal axis in resistance-trained men. *Nutr Res.* (2013) 33:803–10. doi: 10.1016/j.nutres.2013.07.010
105. Willoughby DS, Spillane M, Schwarz N. Heavy resistance training and supplementation with the alleged testosterone booster nmda has no effect on body composition, muscle performance, and serum hormones associated with the hypothalamo-pituitary-gonadal axis in resistance-trained males. *J Sports Sci Med.* (2014) 13:192–9.
106. Melville GW, Siegler JC, Marshall PW. Three and six grams supplementation of d-aspartic acid in resistance trained men. *J Int Soc Sports Nutr.* (2015) 12:15. doi: 10.1186/s12970-015-0078-7
107. Melville GW, Siegler JC, Marshall PWM. The effects of d-aspartic acid supplementation in resistance-trained men over a three month training period: a randomised controlled trial. *PLoS ONE* (2017) 12:e0182630. doi: 10.1371/journal.pone.0182630
108. Crewther B, Witek K, Zmijewski P, Obminski Z. Short-Term d-Aspartic acid supplementation does not affect serum biomarkers associated with the hypothalamic-pituitary-gonadal axis in male climbers. *Int J Sport Nutr Exerc Metab.* (2018) 21:1–6. doi: 10.1123/ijsnem.2018-0076
109. Katane M, Yamada S, Kawaguchi G, Chinen M, Matsumura M, Ando T, et al. Identification of Novel D-Aspartate oxidase inhibitors by *in silico* screening and their functional and structural characterization *in vitro*. *J Med Chem.* (2015) 58:7328–40. doi: 10.1021/acs.jmedchem.5b00871
110. Fatemi SH, Folsom TD. The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophr Bull.* (2009) 35:528–48. doi: 10.1093/schbul/sbn187
111. Lu L, Mamiya T, Koseki T, Mouri A, Nabeshima T. Genetic animal models of schizophrenia related with the hypothesis of abnormal neurodevelopment. *Biol Pharm Bull.* (2011) 34:1358–63. doi: 10.1248/bpb.34.1358
112. Owen MJ, O'Donovan MC, Thapar A, Craddock N. Neurodevelopmental hypothesis of schizophrenia. *Br J Psychiatry* (2011) 198:173–5. doi: 10.1192/bjp.bp.110.084384
113. Ursini G, Punzi G, Chen Q, Marenco S, Robinson JF, Porcelli A, et al. Convergence of placenta biology and genetic risk for schizophrenia. *Nat Med.* (2018) 24:792–801. doi: 10.1038/s41591-018-0021-y
114. Ikonomidou C, Bittigau P, Koch C, Genz K, Hoerster F, Felderhoff-Mueser U, et al. Neurotransmitters and apoptosis in the developing brain. *Biochem Pharmacol.* (2001) 62:401–5. doi: 10.1016/S0006-2952(01)00696-7
115. Ritter LM, Vazquez DM, Meador-Woodruff JH. Ontogeny of ionotropic glutamate receptor subunit expression in the rat hippocampus. *Brain Res Dev Brain Res.* (2002) 139:227–36. doi: 10.1016/S0165-3806(02)00572-2
116. Nacher J, McEwen BS. The role of N-methyl-D-asparate receptors in neurogenesis. *Hippocampus* (2006) 16:267–70. doi: 10.1002/hipo.20160

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 06 November 2018
doi: 10.3389/fpsyt.2018.00566

Combination of *G72* Genetic Variation and G72 Protein Level to Detect Schizophrenia: Machine Learning Approaches

Eugene Lin[1,2,3†], *Chieh-Hsin Lin*[3,4,5†], *Yi-Lun Lai*[3], *Chiung-Hsien Huang*[6], *Yu-Jhen Huang*[7] *and Hsien-Yuan Lane*[3,7,8,9*]

[1] Department of Electrical & Computer Engineering, University of Washington, Seattle, WA, United States, [2] Department of Biostatistics, University of Washington, Seattle, WA, United States, [3] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, [4] Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan, [5] School of Medicine, Chang Gung University, Taoyuan, Taiwan, [6] Department of Medicine Research, China Medical University Hospital, Taichung, Taiwan, [7] Department of Psychiatry, China Medical University Hospital, Taichung, Taiwan, [8] Brain Disease Research Center, China Medical University Hospital, Taichung, Taiwan, [9] Department of Psychology, College of Medical and Health Sciences, Asia University, Taichung, Taiwan

OPEN ACCESS

Edited by:
Chad A. Bousman,
University of Calgary, Canada

Reviewed by:
Jingyu Liu,
Mind Research Network (MRN), United States
Tianmei Si,
Peking University Sixth Hospital, China

***Correspondence:**
Hsien-Yuan Lane
hylane@gmail.com

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *16 August 2018*
Accepted: *18 October 2018*
Published: *06 November 2018*

Citation:
Lin E, Lin C-H, Lai Y-L, Huang C-H, Huang Y-J and Lane H-Y (2018) Combination of G72 Genetic Variation and G72 Protein Level to Detect Schizophrenia: Machine Learning Approaches. Front. Psychiatry 9:566. doi: 10.3389/fpsyt.2018.00566

The *D-amino acid oxidase activator* (*DAOA*, also known as *G72*) gene is a strong schizophrenia susceptibility gene. Higher G72 protein levels have been implicated in patients with schizophrenia. The current study aimed to differentiate patients with schizophrenia from healthy individuals using *G72* single nucleotide polymorphisms (SNPs) and G72 protein levels by leveraging computational artificial intelligence and machine learning tools. A total of 149 subjects with 89 patients with schizophrenia and 60 healthy controls were recruited. Two *G72* genotypes (including rs1421292 and rs2391191) and G72 protein levels were measured with the peripheral blood. We utilized three machine learning algorithms (including logistic regression, naive Bayes, and C4.5 decision tree) to build the optimal predictive model for distinguishing schizophrenia patients from healthy controls. The naive Bayes model using two factors, including *G72* rs1421292 and G72 protein, appeared to be the best model for disease susceptibility (sensitivity = 0.7969, specificity = 0.9372, area under the receiver operating characteristic curve (AUC) = 0.9356). However, a model integrating *G72* rs1421292 only slightly increased the discriminative power than a model with G72 protein alone (sensitivity = 0.7941, specificity = 0.9503, AUC = 0.9324). Among the three models with G72 protein alone, the naive Bayes with G72 protein alone had the best specificity (0.9503), while logistic regression with G72 protein alone was the most sensitive (0.8765). The findings remained similar after adjusting for age and gender. This study suggests that G72 protein alone, without incorporating the two *G72* SNPs, may have been suitable enough to identify schizophrenia patients. We also recommend applying both naive Bayes and logistic regression models for the best specificity and sensitivity, respectively. Larger-scale studies are warranted to confirm the findings.

Keywords: artificial intelligence, D-amino acid oxidase activator, G72, machine learning algorithm, schizophrenia, single nucleotide polymorphism

INTRODUCTION

Schizophrenia is a severe mental disorder characterized by symptoms such as delusions, hallucinations, blunted affect, impaired executive function, reduced motivation, and disorganized communication (1). The prevalence of schizophrenia is around 1% worldwide, and the social and economic costs of schizophrenia are enormous (2, 3). Converging evidence from genome-wide linkage studies, genetic case-control association studies, and genome-wide association studies indicate that several potential candidate genes are associated with schizophrenia (4). More and more genetic studies have employed novel computational tools such as naive Bayes to conduct gene discovery and detect new gene loci associated with schizophrenia (5). Identification of susceptibility genes for schizophrenia will help in early detection and prevention of high-risk individuals, as well as in developing novel therapies (6). The *D-amino-acid oxidase activator* (*DAOA*, also named *G72*) gene is one of the candidate genes.

The *G72* gene, located on chromosome 13q3, exists in exclusively four primate species (7). Furthermore, the *G72* gene encodes the protein that has been shown to function as a putative activator of D-amino acid oxidase (DAO), located in peroxisomes (7) and a mitochondrial protein (8). *In vitro* studies also demonstrate that the G72 protein binds to and activates DAO, which is capable of oxidizing D-amino acids such as D-serine, an agonist of the N-methyl-D-aspartate receptor (NMDAR) (7, 9). The agonist activity at NMDAR may have particular relevance to a novel drug target for treatment of schizophrenia (10–16). One hypothesis of schizophrenia is that individuals who overproduce the G72 protein have lower D-amino acid levels and reduced NMDAR activity, predisposing them to schizophrenia (17, 18). A study suggests that the plasma G72 protein levels may be distinctively higher in patients with schizophrenia than healthy individuals (18). Of note, G72 protein levels are very similar between the medicated patients and the drug-free patients, implying that antipsychotic treatment does not influence G72 levels in plasma (18). In addition, *G72* transgenic mice studies indicate a role of *G72* in modulating behaviors relevant to schizophrenia (19–21). The *G72* gene was also reported to predispose to schizophrenia in French Canadian (7), Russian (7), Chinese (22–24), German (25), and Ashkenazi (26) populations in single nucleotide polymorphism (SNP)-based studies.

A pilot study (18) modeled disease susceptibility to schizophrenia with plasma G72 protein levels using logistic regression. The current larger-sized study compared three artificial intelligence and machine learning techniques (including logistic regression, naive Bayes, and C4.5 decision tree) in predicting schizophrenia using G72 protein levels plus *G72* SNPs. These three artificial intelligence and machine learning algorithms were chosen because they are well-known techniques with distinctively representational models; regression models for logistic regression (27), probabilistic models for naive Bayes (28), and decision tree models for the C4.5 algorithm (29).

MATERIALS AND METHODS

Study Population

This study was approved by the institutional review board of China Medical University Hospital, Taiwan, and carried out in accordance with the Declaration of Helsinki. Consecutive patients were screened and recruited from the psychiatric treatment programs of China Medical University Hospital, which is a major medical center in Taiwan. The patient population is similar to that of other mental health facilities. After complete description of the study to the subjects, written informed consents were obtained in line with the institutional review board guidelines. The study subjects were partially original to a previous study (18); the same 60 healthy individuals, but with more schizophrenia patients.

In the cohort, both patients and controls were Han Chinese aged 18–50 years, who were physically and neurologically healthy and had normal laboratory assessments (including urine/blood routine and biochemical tests). Both patients and controls were evaluated by the research psychiatrists using the Structured Clinical Interview for DSM-IV (SCID) for diagnosis. All patients had a DSM-IV diagnosis of schizophrenia. Patients with Axis I diagnosis other than schizophrenia, or any Axis II diagnosis were not included. All healthy volunteers were free of any Axis I or II psychiatric disorder. To exclude potential confounding effects, all participants were non-smokers and had no DSM-IV diagnosis of substance (including alcohol) abuse or dependence.

Drug history was ascertained by interviewing the patients and family members or caregivers, contacting other health care providers, and reviewing chart. Healthy controls had no history of exposure to psychotropic agents. Among schizophrenia patients, some patients were psychotropic-free for 3 months or longer and the other patients were stabilized on antipsychotics (risperidone, zotepine, haloperidol, quetiapine, amisulpride, sulpiride, flupentixol, olanzapine, ziprasidone, chlorpromazine, or paliperidone) for at least 3 months (18). The G72 protein level was not correlated with the medications administrated by patients (18).

Laboratory Assessments: Genotyping

DNA was isolated from blood samples using MasterPure DNA purification kit following the manufacturer's instructions (EPICENTRE, Madison, Wisconsin, USA). To extract DNA, we used 200 μl of blood which was further solved in 100 μl of distilled water (30). The extracted DNA was diluted to the concentration of 50 ng/μl determined by the absorbance at 260 nM (ND-1000 UV-Vis spectrophotometer, Thermo Fisher Scientific Inc.). Four standard DNA samples with known genotypes were used for quality control (31).

All SNP genotyping was performed using the Taqman SNP genotyping assay (ABI: Applied Biosystems Inc., Foster City, CA, USA). The primers and probes of SNPs were provided by the ABI Company. The PCR reaction was conducted in 15 μl reaction volume which contained 0.4 μl DNA sample (50 ng), 7.5 μl Master mix (Roche), and 0.4 μl 40x primer pairs and probes. The samples were pre-incubated at 95°C for 10 min to activate the Hot-Start DNA polymerase and to denature DNA, following

by 40 amplification cycles of 92°C denaturation for 15 s and 60°C for 60 s. The probe fluorescence signal detection was performed using the ABI Prism 7500 Real-Time PCR System.

Laboratory Assessments: Western Blotting

The plasma G72 protein expression levels were examined by western blotting (18). Ten milliliter of blood was collected into EDTA-containing blood collection tubes by personnel trained in phlebotomy using sterile technique. The blood specimens were processed immediately by centrifugation at 500 g. After centrifugation, plasma was quickly dissected and immediately stored at −80°C until western blotting.

For western blotting, 100 μl plasma was depleted using ProteoPrep® Blue Albumin and IgG Depletion Kit (Sigma). The low-abundant protein fractions were collected to 100 μl. Then, 10 μl of the fractions were mixed with 4X sample buffer (500 mM Tris-HCl (pH 6.8), 16% SDS, 80% glycerol, 400 mM DTT, and 0.08% bromophenol blue) and separated on 12% SDS-PAGE. Proteins in the gels were transferred to 0.45 μm polyvinylidene difluoride (PVDF) membrane (Millipore). The membranes were placed in 5% nonfat dry milk in TBST (20 mM Tris-HCl pH 7.6, 500 mM sodium chloride, 0.1% Tween 20) for 1 h at room temperature, then incubated with goat anti-G72 antibody (G72(N15):sc-46118, Santa Cruz Biotechnology) diluted by 1:1,000 in TBST overnight at 4°C. The membranes were washed for 3 times in TBST and incubated for 2 h with a HRP-linked anti-goat IgG secondary antibody (sc-2030, Santa Cruz Biotechnology) diluted by 1:5,000 in TBST. After 3 washes in TBST, the blots were visualized with an ECL Advance Western Blotting Detection Kit (RPN2135, GE Healthcare). The stained membranes were photographed on ImageQuant LAS 4000 mini (GE Healthcare) and quantified using ImageQuant™ TL 7.0 software (GE Healthcare) by measuring the relative intensity from each band and normalized to the G72 recombinant protein (20 ng) signals. All western blot analyses were repeated for two times.

Machine Learning Algorithms

Machine learning algorithm is a procedure for choosing the best hypothesis from a set of alternatives that fit a set of observations (27, 32). The advantages of machine learning algorithms, including nonlinearity, fault tolerance, and real-time operation, make them suitable for complex applications (33). The current study employed three families of machine learning algorithms, including logistic regression, naive Bayes, and C4.5 decision tree. Logistic regression analysis, the standard method for clinical classification (27), was used as a basis for comparison. The analyses were performed using the Waikato Environment for Knowledge Analysis (WEKA) software (27).

The naive Bayes classifier assumes that the presence or absence of a particular feature is unrelated to the presence or absence of any other feature (27). It calculates the probability that a given instance belongs to a certain class ("schizophrenia" or "control" in this study) by using Bayes' theorem.

The C4.5 decision tree is a model which builds decision trees top-down and prunes them using the concept of information entropy (27). The tree is first constructed by finding the root node (SNP or protein level) that is most discriminative for differentiating a disease status from "control." The best single feature test is decided by the information gain from choosing a feature (SNP or protein level) to split the data into subsets. Here, we used the default parameters of WEKA, such as 0.25 for the confidence factor and 2 for the minimum number of instances per leaf node (34).

Evaluation of the Predictive Performance

The repeated 10-fold cross-validation method was used to investigate the generalization of the predictive models produced by the aforementioned algorithms (34–36). To measure the performance of the predictive models, we used the receiver operating characteristic (ROC) method and calculated the area under curve (AUC) to compare the performances of different predictive models (34, 35). AUC is a better performance metric than accuracy; the higher AUC means the better performance (36).

Statistical Analysis

We analyzed the categorical data using the chi-square test. Differences for continuous variables were compared using the Student's *t*-test (37). Genotype frequencies were evaluated for Hardy-Weinberg equilibrium using a χ^2 goodness-of-fit test. The criterion for significance was set at $P < 0.05$ for all tests. Data are presented as mean ± standard deviation (SD).

RESULTS

Findings From the Unmatched Sample

The participants were 60 unrelated healthy individuals and 89 schizophrenia patients. As shown in **Table 1**, there was no significant difference in gender distribution between the two groups. The mean age (37.8 ± 10.5) of schizophrenia patients was older than that of healthy controls (32.8 ± 9.9, $P = 0.004$). The mean level of G72 protein in the plasma of schizophrenia patients was markedly higher than that of healthy controls (4.057 ± 2.594 ng/μL vs. 1.147 ± 0.574 ng/μL, respectively, $P < 0.0001$) (**Table 1**). The genotype frequencies for both rs1421292 and rs2391191 of the *G72* gene were in Hardy–Weinberg equilibrium ($P = 0.39$ and 0.27, respectively).

AUC After Adding rs1421292 Was Only Slightly Better Than That From G72 Protein Alone

Table 2 summarizes the results from the naive Bayes algorithm. We generated five models (Models 1–5) with various combinations of three factors (rs2391191, rs1421292, and G72 protein levels). Among the five models, Model 2 with rs1421292 and G72 protein levels had the best AUC. Its AUC, sensitivity, and specificity were 0.9356, 0.7969, and 0.9372, respectively (**Table 2**). However, the AUC value after adding rs1421292 was only slightly better than that of G72 protein alone by 0.32% (Model 2 vs. Model 5).

We then employed the C4.5 decision tree algorithm with the same three factors (**Table 3**). Among the five models, Model 7 with rs1421292 and G72 protein levels had the best AUC. Its AUC, sensitivity, and specificity were 0.8525, 0.8202, and 0.8843,

TABLE 1 | Demographic characteristics of schizophrenia patients and unmatched healthy individuals.

Parameter	Healthy individuals	Schizophrenia patients	*P*-value [a]
N	60	89	
Gender			0.825
Male	36 (61.9%)	55 (70.4%)	
Female	24 (38.1%)	34 (29.6%)	
Age (year), mean (SD)	32.8 ± 9.9	37.8 ± 10.5	0.004
Education (year)	15.1 ± 2.2	11.5 ± 2.0	<0.0001
Age at onset (year)		22.9 ± 6.1	
Illness duration (m)		169.3 ± 109.3	
PANSS total score		94.8 ± 18.6	
G72 level (ng/μL)	1.147 ± 0.574	4.057 ± 2.594	<0.0001

PANSS: Positive and Negative Syndrome Scale.
[a]*Chi-square test for the categorical data; Student's t-test for continuous variables.*

TABLE 2 | Five naive Bayes models for differentiating schizophrenia patients from unmatched healthy individuals.

Model	AUC	Sensitivity	Specificity	Number of factors
(1) Using G72 protein, rs1421292, rs2391191	0.9280	0.7945	0.9213	3
(2) Using G72 protein, rs1421292	0.9356	0.7969	0.9372	2
(3) Using G72 protein, rs2391191	0.9244	0.7924	0.9320	2
(4) Using rs1421292, rs2391191	0.4612	0.9704	0.0070	2
(5) Using G72 protein	0.9324	0.7941	0.9503	1

respectively (**Table 3**). The AUC value after adding rs1421292 was only slightly better than that of G72 protein alone by 0.19% (Model 7 vs. Model 10).

We finally tested the same factors with logistic regression (**Table 4**). The AUC, sensitivity, and specificity for the best logistic regression model (Model 12, applying rs1421292, and G72 protein levels) were 0.9272, 0.8576, and 0.8923, respectively. The AUC value only slightly increased by 0.97% after adding rs1421292 (Model 12 vs. Model 15).

Of the G72 Protein Models, Naive Bayes Was Specific; And Logistic Regression, Sensitive

Among all the 15 models (Models 1–15) with unmatched schizophrenia patients and healthy controls, the naive Bayes (Model 2) with rs1421292 and G72 protein levels had the highest AUC. Of the three models with G72 protein alone (Models 5, 10, and 15), the naive Bayes (Model 5) had the best specificity (0.9503) and logistic regression (Model 15) had the best sensitivity (0.8765).

We further tested the relationship between *G72* genotypes and G72 protein levels. The distribution of the two SNPs [for example, the numbers of TT ($n = 57$), TA ($n = 65$), and AA ($n = 27$) carriers in rs1421292] was illustrated in **Table 5**. As shown in **Table 5**, the G72 protein levels were marginally higher in the subjects with the TT or TA genotype than the AA homozygotes for rs1421292 (3.051 ± 2.588 vs. 2.137 ± 1.819; $P = 0.084$). There was no association between genetic variances of rs2391191 and G72 protein levels.

TABLE 3 | Five C4.5 decision tree models for differentiating schizophrenia patients from unmatched healthy individuals.

Model	AUC	Sensitivity	Specificity	Number of factors
(6) Using G72 protein, rs1421292, rs2391191	0.8515	0.8236	0.8772	3
(7) Using G72 protein, rs1421292	0.8525	0.8202	0.8843	2
(8) Using G72 protein, rs2391191	0.8504	0.8275	0.8725	2
(9) Using rs1421292, rs2391191	0.5000	1.0000	0.0000	2
(10) Using G72 protein	0.8506	0.8274	0.8725	1

TABLE 4 | Five logistic regression models for differentiating schizophrenia patients from unmatched healthy individuals.

Model	AUC	Sensitivity	Specificity	Number of factors
(11) Using G72 protein, rs1421292, rs2391191	0.9200	0.8567	0.8400	3
(12) Using G72 protein, rs1421292	0.9272	0.8576	0.8923	2
(13) Using G72 protein, rs2391191	0.9107	0.8713	0.8607	2
(14) Using rs1421292, rs2391191	0.4533	0.9619	0.0088	2
(15) Using G72 protein	0.9175	0.8765	0.8577	1

Findings From the Matched Sample

Next, we selected 66 patients from the schizophrenia group to match better with healthy controls by age. The demographic characteristics of age and gender-matched schizophrenia patients and healthy controls are shown in **Table 6**. There was no significant difference in gender and age distributions between the two groups. The G72 levels in the plasma of schizophrenia patients were markedly higher than that of the matched healthy controls (4.188 ± 2.772 ng/μL and 1.147 ± 0.574 ng/μL, respectively, $P < 0.0001$) (**Table 6**).

The Findings From the Matched Sample Were Similar to Those From the Unmatched Sample

Table 7 shows the analytic results of schizophrenia patients and matched healthy controls. The findings from the matched sample were similar to those from the unmatched sample. Among the three models (Models 16–18) with G72 protein alone, the naive Bayes model (Model 16) performed best in specificity (0.966), and logistic regression (Model 18) had the best sensitivity (0.8483) (**Table 7**).

TABLE 5 | Relationship between *G72* genotypes and G72 protein level with schizophrenia patients and unmatched healthy individuals.

***G72* rs1421292**	**AA**	**TT**	**TA**	**TT + TA**	***P*-value[a]**	***P*-value[b]**	***P*-value[c]**
N	27	57	65	122			
G72 protein level, mean (SD)	2.137±1.819	3.219±3.032	2.903±2.139	3.051±2.588	0.09	0.11	0.084
***G72* rs2391191**	**AA**	**GG**	**AG**	**GG + AG**	***P*-value[d]**	***P*-value[e]**	***P*-value[f]**
N	63	22	64	86			
G72 protein level, mean (SD)	2.586±2.083	3.570±3.356	2.943±2.499	3.104±2.736	0.11	0.38	0.21

[a] *P value for comparing the subjects of the AA genotype with those of the TT genotype.*
[b] *P value for comparing the subjects of the AA genotype with those of the TA genotype.*
[c] *P value for comparing the subjects of the AA genotype with those of the TT or TA genotype.*
[d] *P value for comparing the subjects of the AA genotype with those of the GG genotype.*
[e] *P value for comparing the subjects of the AA genotype with those of the AG genotype.*
[f] *P value for comparing the subjects of the AA genotype with those of the GG or AG genotype.*

TABLE 6 | Demographic characteristics of schizophrenia patients and matched healthy individuals.

Parameter	**Healthy individuals**	**Schizophrenia patients**	***P*-value [a]**
N	60	66	
Gender			0.783
Male	36 (61.9%)	38 (57.6%)	
Female	24 (38.1%)	28 (42.4%)	
Age (year), mean (SD)	32.8 ± 9.9	33.2 ± 7.2	0.820
Education (year)	15.1 ± 2.2	11.6 ± 2.0	<0.0001
Age at onset (year)		21.3 ± 5.5	
Illness duration (m)		141.1 ± 95.7	
PANSS total score		95.9 ± 19.3	
G72 level (ng/μL)	1.147 ± 0.574	4.188 ± 2.772	<0.0001

PANSS: Positive and Negative Syndrome Scale.
[a] *Chi-square test for the categorical data; Student's t-test for continuous variables.*

TABLE 7 | The models of naive Bayes, C4.5 decision tree, and Logistic regression for differentiating schizophrenia patients from matched healthy individuals using G72 protein.

Model	**AUC**	**Sensitivity**	**Specificity**	**Number of factors**
(16) Naive Bayes with G72 protein	0.9396	0.7914	0.9660	1
(17) C4.5 decision tree with G72 protein	0.8510	0.7871	0.9152	1
(18) Logistic regression with G72 protein	0.9099	0.8483	0.9072	1

DISCUSSION

To our knowledge, this is the first study to examine the relationships between schizophrenia and *G72* SNPs plus plasma G72 protein levels. We compared three machine learning algorithms, including logistic regression, naive Bayes, and C4.5 decision tree, in differentiating schizophrenia patients from healthy individuals. The results showed that the naive Bayes with *G72* rs1421292 SNP and G72 protein levels (Model 2) performed best among all models (Models 1–15). The combination of *G72* rs1421292 SNP and G72 protein levels was also the best model using the C4.5 decision tree (Model 7) and logistic regression (Model 12). These results were consistent with another finding of this study; that is, *G72* rs1421292 SNP was marginally associated with G72 protein levels (**Table 5**). The proposed procedures can be implemented using the publicly available software WEKA (27) and thus can be widely used in genomic studies.

However, the AUC value after adding rs1421292 was only slightly better than that of G72 protein alone by an increase of 0.32% (Model 2 vs. Model 5) and 0.97% (Model 12 vs. Model 15), respectively. Hence, the present study suggests that G72 protein alone may have been feasible enough in AUC. Moreover, among the three models with G72 protein alone, logistic regression performed best in sensitivity, and the naive Bayes model was the most specific. This finding remained similar in the matched sample. We therefore recommend a combination model using logistic regression (for sensitivity) and naive Bayes (for specificity).

The common SNPs, such as rs2391191 and rs1421292, of the *G72* gene have received considerable attention. These two SNPs were shown to be associated with schizophrenia (7, 23–25); however, the findings are discordant. The rs2391191 SNP was reported to predispose to schizophrenia in Chinese (23, 24) and German (25) subjects, but not in French Canadian (7), United States (38), Scottish (22), Chinese (22, 39), and Taiwanese (40) populations. On the other hand, the rs1421292 SNP was found to be associated with schizophrenia in French Canadian (7), Russian (7), and German (25) subjects, but not in Japanese sample (41), UK population (42) and a mix of different races (including 84% Caucasian and 9% African American) in the United States (38). Moreover, two recent genome-wide association studies (GWAS) have been conducted to identify susceptible genetic loci affecting schizophrenia in mainly European (43) and Chinese (44) populations, respectively. However, no association of schizophrenia with SNPs in the *G72* gene was found in these two large GWAS. Furthermore, by utilizing expression quantitative trait loci (eQTL) analyses, one of these GWAS implicated that there was no genetic risk regulating gene expression of *G72* effect in brain or blood when eQTL

analyses were used to explain associations with schizophrenia (43). The current study showed that the models which combined rs2391191 and rs1421292 (Models 4, 9, and 14) were not as good as other models (**Tables 2–4**). Moreover, adding rs2391191 or rs1421292 could not increase the AUC significantly than G72 protein alone. In agreement with several previous studies (7, 22, 38–44), the current study with a small sample size didn't demonstrate an association between the two *G72* SNPs and schizophrenia.

In our previous study (18) on G72 protein levels, the severity of disease, the medications administrated by patients, as well as illness duration of the medicated patients did not influence the G72 protein level. In addition, the G72 protein level was significantly associated with schizophrenia in multivariate logistic regression analyses (18). The G72 protein level was also higher in drug-free or medicated schizophrenia patients than in healthy controls (18). The current larger-sized study extended the previous study by combining G72 protein levels with *G72* SNPs as well as by leveraging the state-of-the-art artificial intelligence and machine learning algorithms. The current results implicated that the relevance of G72 protein levels to schizophrenia is much more significant than that of SNPs.

This study has several limitations. First, we chose only two SNPs of *G72* for the current study because they seem to be two most commonly used SNPs. Whether other SNPs of *G72* (25) could contribute more in the models of predicting schizophrenia remains unknown. Second, the findings of the current study came from a single population. More studies are necessary to testify whether the findings could be replicated in non-Taiwanese subjects (45, 46). Third, the small sample size does not allow us to draw definite conclusions (46). In the future, large-scale prospective studies in other ethnicities are warranted to reconfirm the potential of G72 protein level and *G72* SNPs as the biomarkers for schizophrenia.

CONCLUSIONS

In conclusion, this preliminary study tested and compared numerous models using machine learning algorithms for predicting schizophrenia. The findings suggest that the models with G72 protein alone, without adding *G72* SNPs, may have good enough power to discriminate patients with schizophrenia from healthy individuals. We also propose a combination of logistic regression and naive Bayes models to build a both sensitive and specific model to predict schizophrenia. Independent replications with larger-scale studies in other racial populations are needed to confirm the role of the *G72* SNPs and G72 protein found in the current study.

AUTHOR CONTRIBUTIONS

EL, C-HL, and H-YL designed the study. EL analyzed the data. EL, C-HL, and H-YL drafted and revised the manuscript. Y-LL, Y-JH, and C-HH conducted the laboratory experiments. All authors provided the final approval of the version to be published.

FUNDING

This work was supported by National Health Research Institutes, Taiwan (NHRI-EX107-10731NI), Ministry of Science and Technology in Taiwan (MOST 107-2314-B-039—039), Taiwan Ministry of Health and Welfare Clinical Trial, and Research Center of Excellence (MOHW107-TDU-B-212-123004), China Medical University and Hospital (DMR-103-085; CMU107-BC-4).

REFERENCES

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. *PLoS Med.* (2005) 2:e141. doi: 10.1371/journal.pmed.0020141
2. Messias EL, Chen CY, Eaton WW. Epidemiology of schizophrenia: review of findings and myths. *Psychiatr Clin North Am.* (2007) 30:323–38. doi: 10.1016/j.psc.2007.04.007
3. Patel A, Everitt B, Knapp M, Reeder C, Grant D, Ecker C, et al. Schizophrenia patients with cognitive deficits: factors associated with costs. *Schizophr Bull* (2006) 32:776–85. doi: 10.1093/schbul/sbl013
4. Sullivan PF. How good were candidate gene guesses in schizophrenia genetics? *Biol Psychiatry* (2017) 82:696–7. doi: 10.1016/j.biopsych.2017.09.004
5. Andreassen OA, Thompson WK, Dale AM. Boosting the power of schizophrenia genetics by leveraging new statistical tools. *Schizophr Bull.* (2014) 40:13–7. doi: 10.1093/schbul/sbt168
6. Jarskog LF, Miyamoto S, Lieberman JA. Schizophrenia: new pathological insights and therapies. *Annu Rev Med.* (2007) 58:49–61. doi: 10.1146/annurev.med.58.060904.084114
7. Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, et al. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. *Proc Natl Acad Sci USA.* (2002) 99:13675–80. doi: 10.1073/pnas.182412499
8. Kvajo M, Dhilla A, Swor DE, Karayiorgou M, Gogos JA. Evidence implicating the candidate schizophrenia/bipolar disorder susceptibility gene G72 in mitochondrial function. *Mol Psychiatry* (2008) 13:685–96. doi: 10.1038/sj.mp.4002052
9. Sacchi S, Bernasconi M, Martineau M, Mothet JP, Ruzzene M, Pilone MS, et al. pLG72 modulates intracellular D-serine levels through its interaction with D-amino acid oxidase: effect on schizophrenia susceptibility. *J Biol Chem.* (2008) 283:22244–56. doi: 10.1074/jbc.M709153200
10. Coyle JT, Tsai G, Goff D. Converging evidence of NMDA receptor hypofunction in the pathophysiology of schizophrenia. *Ann N Y Acad Sci.* (2003) 1003:318–27. doi: 10.1196/annals.1300.020
11. Ermilov M, Gelfin E, Levin R, Lichtenberg P, Hashimoto K, Javitt DC, et al. A pilot double-blind comparison of d-serine and high-dose olanzapine in treatment-resistant patients with schizophrenia. *Schizophr Res.* (2013) 150:604–5. doi: 10.1016/j.schres.2013.09.018
12. Goff DC. D-cycloserine: an evolving role in learning and neuroplasticity in schizophrenia. *Schizophr Bull* (2012) 38:936–41. doi: 10.1093/schbul/sbs012
13. Javitt DC. Twenty-five years of glutamate in schizophrenia: are we there yet? *Schizophr Bull.* (2012) 38:911–3. doi: 10.1093/schbul/sbs100
14. Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* (2012) 37:4–15. doi: 10.1038/npp.2011.181
15. Lane HY, Lin CH, Green MF, Hellemann G, Huang CC, Chen PW, et al. Add-on treatment of benzoate for schizophrenia: a randomized, double-blind, placebo-controlled trial of D-amino acid oxidase inhibitor. *JAMA Psychiatry* (2013) 70:1267–75. doi: 10.1001/jamapsychiatry.2013.2159

16. Lin CH, Lin CH, Chang YC, Huang YJ, Chen PW, Yang HT, et al. Sodium benzoate, a D-amino acid oxidase inhibitor, added to clozapine for the treatment of schizophrenia: a randomized, double-blind, placebo-controlled trial. *Biol Psychiatry* (2017) 84:422–32. doi: 10.1016/j.biopsych.2017.12.006
17. Hashimoto K, Fukushima T, Shimizu E, Komatsu N, Watanabe H, Shinoda N, et al. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. *Arch Gen Psychiatry* (2003) 60:572–6. doi: 10.1001/archpsyc.60.6.572
18. Lin CH, Chang HT, Chen YJ, Lin CH, Huang CH, Tun R, et al. Distinctively higher plasma G72 protein levels in patients with schizophrenia than in healthy individuals. *Mol Psychiatry* (2014) 19:636–7. doi: 10.1038/mp.2013.80
19. Cheng L, Hattori E, Nakajima A, Woehrle NS, Opal MD, Zhang C, et al. Expression of the G72/G30 gene in transgenic mice induces behavioral changes. *Mol Psychiatry* (2014) 19:175–83. doi: 10.1038/mp.2012.185
20. Otte DM, Bilkei-Gorzo A, Filiou MD, Turck CW, Yilmaz O, Holst MI, et al. Behavioral changes in G72/G30 transgenic mice. *Eur Neuropsychopharmacol.* (2009) 19:339–48. doi: 10.1016/j.euroneuro.2008.12.009
21. Otte DM, Sommersberg B, Kudin A, Guerrero C, Albayram O, Filiou MD, et al. N-acetyl cysteine treatment rescues cognitive deficits induced by mitochondrial dysfunction in G72/G30 transgenic mice. *Neuropsychopharmacology* (2011) 36:2233–43. doi: 10.1038/npp.2011.109
22. Ma J, Qin W, Wang XY, Guo TW, Bian L, Duan SW, et al. Further evidence for the association between G72/G30 genes and schizophrenia in two ethnically distinct populations. *Mol Psychiatry* (2006) 11:479–87. doi: 10.1038/sj.mp.4001788
23. Wang X, He G, Gu N, Yang J, Tang J, Chen Q, et al. Association of G72/G30 with schizophrenia in the Chinese population. *Biochem Biophys Res Commun.* (2004) 319:1281–6. doi: 10.1016/j.bbrc.2004.05.119
24. Zou F, Li C, Duan S, Zheng Y, Gu N, Feng G, et al. A family-based study of the association between the G72/G30 genes and schizophrenia in the Chinese population. *Schizophr Res.* (2005) 73:257–61. doi: 10.1016/j.schres.2004.01.015
25. Schumacher J, Jamra RA, Freudenberg J, Becker T, Ohlraun S, Otte AC, et al. Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. *Mol Psychiatry* (2004) 9:203–7. doi: 10.1038/sj.mp.4001421
26. Korostishevsky M, Kaganovich M, Cholostoy A, Ashkenazi M, Ratner Y, Dahary D, et al. Is the G72/G30 locus associated with schizophrenia? single nucleotide polymorphisms, haplotypes, and gene expression analysis. *Biol Psychiatry* (2004) 56:169–76. doi: 10.1016/j.biopsych.2004.04.006
27. Witten IHFE. *Data Mining: Practical Machine Learning Tools and Techniques*. Francisco, CA: Morgan Kaufmann Publishers (2005).
28. Lee KE, Sha N, Dougherty ER, Vannucci M, Mallick BK. Gene selection: a Bayesian variable selection approach. *Bioinformatics* (2003) 19:90–7. doi: 10.1093/bioinformatics/19.1.90
29. Hewett R, Kijsanayothin P. Tumor classification ranking from microarray data. *BMC Genomics* (2008) 9(Suppl 2):S21. doi: 10.1186/1471-2164-9-S2-S21
30. Hsiao TJ, Lin E. The Pro12Ala polymorphism in the peroxisome proliferator-activated receptor gamma (PPARG) gene in relation to obesity and metabolic phenotypes in a Taiwanese population. *Endocrine* (2015) 48:786–93. doi: 10.1007/s12020-014-0407-7
31. Hsiao TJ, Lin E. The ENPP1 K121Q polymorphism is associated with type 2 diabetes and related metabolic phenotypes in a Taiwanese population. *Mol Cell Endocrinol.* (2016) 433:20–5. doi: 10.1016/j.mce.2016.05.020
32. Lin E, Kuo PH, Liu YL, Yu YW, Yang AC, Tsai SJ. A deep learning approach for predicting antidepressant response in major depression using clinical and genetic biomarkers. *Front Psychiatry* (2018) 9:290. doi: 10.3389/fpsyt.2018.00290
33. Lin E, Lane HY. Machine learning and systems genomics approaches for multi-omics data. *Biomark Res.* (2017) 5:2. doi: 10.1186/s40364-017-0082-y
34. Huang LC, Hsu SY, Lin E. A comparison of classification methods for predicting Chronic Fatigue Syndrome based on genetic data. *J Transl Med.* (2009) 7:81. doi: 10.1186/1479-5876-7-81
35. Lin E, Hwang Y. A support vector machine approach to assess drug efficacy of interferon-α and ribavirin combination therapy. *Mol Diagn Ther.* (2008) 12:219–23. doi: 10.1007/BF03256287
36. Linden A. Measuring diagnostic and predictive accuracy in disease management: an introduction to receiver operating characteristic (ROC) analysis. *J Eval Clin Pract.* (2006) 12:132–9. doi: 10.1111/j.1365-2753.2005.00598.x
37. Lin E, Kuo PH, Liu YL, Yang AC, Tsai SJ. Transforming growth factor-beta signaling pathway-associated genes SMAD2 and TGFBR2 are implicated in metabolic syndrome in a Taiwanese population. *Sci Rep.* (2017) 7:13589. doi: 10.1038/s41598-017-14025-4
38. Mulle JG, Chowdari KV, Nimgaonkar V, Chakravarti A. No evidence for association to the G72/G30 locus in an independent sample of schizophrenia families. *Mol Psychiatry* (2005) 10:431–3. doi: 10.1038/sj.mp.4001619
39. Yue W, Kang G, Zhang Y, Qu M, Tang F, Han Y, et al. Association of DAOA polymorphisms with schizophrenia and clinical symptoms or therapeutic effects. *Neurosci Lett.* (2007) 416:96–100. doi: 10.1016/j.neulet.2007.01.056
40. Liu YL, Fann CS, Liu CM, Chang CC, Wu JY, Hung SI, et al. No association of G72 and D-amino acid oxidase genes with schizophrenia. *Schizophr Res.* (2006) 87:15–20. doi: 10.1016/j.schres.2006.06.020
41. Ohi K, Hashimoto R, Yasuda Y, Yoshida T, Takahashi H, Iike N, et al. Association study of the G72 gene with schizophrenia in a Japanese population: a multicenter study. *Schizophr Res.* (2009) 109:80–5. doi: 10.1016/j.schres.2009.01.019
42. Bass NJ, Datta SR, McQuillin A, Puri V, Choudhury K, Thirumalai S, et al. Evidence for the association of the DAOA (G72) gene with schizophrenia and bipolar disorder but not for the association of the DAO gene with schizophrenia. *Behav Brain Funct.* (2009) 5:28. doi: 10.1186/1744-9081-5-28
43. Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* (2014) 511:421–7. doi: 10.1038/nature13595
44. Li Z, Chen J, Yu H, He L, Xu Y, Zhang D, et al. Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. *Nat Genet.* (2017) 49:1576–83. doi: 10.1038/ng.3973
45. Lin E, Kuo PH, Liu YL, Yang AC, Kao CF, Tsai SJ. Association and interaction of APOA5, BUD13, CETP, LIPA and health-related behavior with metabolic syndrome in a Taiwanese population. *Sci Rep.* (2016) 6:36830. doi: 10.1038/srep36830
46. Lane HY, Tsai GE, Lin E. Assessing gene-gene interactions in pharmacogenomics. *Mol Diagn Ther.* (2012) 16:15–27. doi: 10.2165/11597270-000000000-00000

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 26 November 2018
doi: 10.3389/fpsyt.2018.00633

Decreased Blood Levels of Oxytocin in Ketamine-Dependent Patients During Early Abstinence

Ming-Chyi Huang[1,2,3], *Lian-Yu Chen*[1,4], *Hu-Ming Chang*[1], *Xiao-Yu Liang*[5], *Chih-Ken Chen*[6,7]*, *Wan-Ju Cheng*[8,9]* and *Ke Xu*[5]

[1] Department of Psychiatry, Taipei City Psychiatric Center, Taipei City Hospital, Taipei, Taiwan, [2] Department of Psychiatry, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [3] Psychiatric Research Center, Taipei Medical University Hospital, Taipei, Taiwan, [4] Institute of Epidemiology and Preventive Medicine, National Taiwan University, Taipei, Taiwan, [5] Department of Psychiatry, Yale University School of Medicine, New Heaven, CT, United States, [6] Department of Psychiatry, Chang Gung Memorial Hospital, Keelung, Taiwan, [7] Chang Gung University School of Medicine, Taoyuan, Taiwan, [8] Department of Psychiatry, China Medical University Hospital, Taichung, Taiwan, [9] Department of Public Health, China Medical University, Taichung, Taiwan

OPEN ACCESS

Edited by:
Kenji Hashimoto, Chiba University, Japan

Reviewed by:
Chun Yang, Huazhong University of Science and Technology, China
Kai Zhang, Chiba University, Japan

***Correspondence:**
Chih-Ken Chen
kenchen@cgmh.org.tw
Wan-Ju Cheng
s871065@gmail.com

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *10 October 2018*
Accepted: *07 November 2018*
Published: *26 November 2018*

Citation:
Huang M-C, Chen L-Y, Chang H-M, Liang X-Y, Chen C-K, Cheng W-J and Xu K (2018) Decreased Blood Levels of Oxytocin in Ketamine-Dependent Patients During Early Abstinence. Front. Psychiatry 9:633. doi: 10.3389/fpsyt.2018.00633

Background: Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, is a common drug of abuse worldwide. Existing evidence suggest a disruption of oxytocin system involves in the development of addiction. In this study, we aimed to investigate the role of oxytocin in ketamine addiction by measuring the blood oxytocin levels in ketamine-dependent (KD) patients.

Methods: Sixty-five KD patients and 65 controls were enrolled. Fasting plasma levels of oxytocin were determined at baseline and 1 and 2 weeks after ketamine withdrawal. Ketamine use variables, Beck Depression Inventory, Beck Anxiety Inventory (BAI), Visual Analog Scale for craving, and Childhood Trauma Questionnaire-short form were assessed in KD patients.

Results: KD patients had significantly lower levels of oxytocin at baseline compared to controls (5.89 ± 2.13 vs. 9.53 ± 4.17 ng/mL, $P < 0.001$). Oxytocin levels increased after one (6.74 ± 2.63, $P < 0.002$) and 2 weeks (6.89 ± 2.69, $P = 0.01$) of withdrawal in KD patient despite the levels were still lower than controls ($P = 0.001$ and 0.002, respectively). The clinical variables did not correlate with baseline oxytocin levels except BAI scores, which showed a negative correlation with the levels ($r = -0.263$; $P = 0.039$).

Conclusion: We found a distinctively reduced oxytocin level in KD patients and the level did not normalize after early abstinence. Lower oxytocin might be associated with anxious phenotype of ketamine dependence. These results suggest that oxytocin system dysregulated following chronic ketamine abuse and might provide insight in evaluating the potential therapeutic use of oxytocin for treating ketamine dependence.

Keywords: oxytocin, ketamine, dependence, withdrawal, NMDA antagonist

INTRODUCTION

Ketamine hydrochloride, a non-competitive antagonist of N-methyl-D-aspartate (NMDA) receptor, has long been used as a short-acting anesthetic agent in humans and veterinary medicine. However, recreational ketamine use has been increased significantly worldwide (1), particularly in East and South-East Asia (2), and constitutes a major challenge to public health (3) because of the association with multi-organ harms (4). Ketamine has become one of the most abused drugs in Taiwan (5). As treatment for ketamine dependence has not yet been available (6), knowledge of the underlying mechanisms that modulates the reward system may provide insight in managing individuals suffering from ketamine dependence.

Emerging evidence suggests that oxytocin is involved in the neuroadaptive processes associated with the development of addiction (7). Oxytocin is a polypeptide hormone synthesized in the magnocellular neurons located within the supraoptic (SON), paraventricular (PVN), and accessory nuclei of the hypothalamus and released by the posterior pituitary gland, acting predominantly upon the oxytocin receptor, which is widely distributed throughout brain circuits related to reward, learning, memory, stress, and addiction (8). Animal studies have demonstrated that oxytocin interferes the development of tolerance to morphine (9) and alcohol (10). It may also have the effects of reducing self-administrative behavior of heroin (9), alcohol (11), methamphetamine (12), and cocaine (13). In reinstatement models of drug relapse, oxytocin was found to suppress the reinstatement of extinguished drug-seeking behavior (13–16). The administration of carbetocin, an oxytocin receptor agonist, prevents reinstatement of conditioned reinforcing effects of morphine (16) and alcohol (14). Further evidence supporting the role of oxytocin in the involvement of addiction is that a negative association of plasma oxytocin levels with novelty-seeking, one of the indicators of addiction, was observed in heroin users (17).

Oxytocin has also been implicated in the regulation of withdrawal symptoms in both animals and humans (18). For example, oxytocin administration attenuates alcohol withdrawal convulsions (19) and blocks the somatic symptoms of nicotine withdrawal (20). In addition, oxytocin has also been reported to ameliorate the severity of acute or protracted opioid withdrawal (9, 16). In human studies, oxytocin was shown to be superior to placebo in reducing alcohol withdrawal symptoms in individuals undergoing alcohol detoxification (21). These observations collectively suggest that oxytocin dysregulation can occur after repeated exposure of drugs of abuse and play a role in the physical and behavioral effects associated with acute drug withdrawal.

Secretion of oxytocin is modulated by several mechanisms, including the regulation via NMDA receptors which are expressed in magnocellular cells of PVN and SON hypothalamic nuclei (22). Oxytocin neurosecretory cells in the nuclei receive a dense glutamatergic innervation, which plays a major role in the control of oxytocin release (23). It has been reported that intraperitoneal injection of a NMDA receptor antagonist (MK-801) decreases plasma concentrations of oxytocin in rats induced by osmotic stimulation (24). MK-801 also impaired the oxytocin responses to emotional stimuli (25). Supporting the link between NMDA and the oxytocinergic systems, one study in zebrafish demonstrated that oxytocin reversed MK-801-induced behavioral deficits in social interaction (26). Therefore, ketamine, as an NMDA antagonist, when being used chronically may alter the expression of oxytocin. To test this hypothesis, this study aimed to explore the difference of oxytocin plasma levels between treatment-seeking ketamine-dependent (KD) patients with the most recent ketamine use in the preceding 24 h and healthy controls. We also explored whether oxytocin level was normalized in the early stage of abstinence in KD patients. Finally, the correlations between oxytocin levels and ketamine use variables (frequency and quantity, craving scores, severity of dependence), depression and anxiety symptoms, and experiences of childhood trauma, which have been associated previously with oxytocin concentrations (27, 28), were also examined.

MATERIALS AND METHODS

Participants

This study was carried out in accordance with the recommendations of Institutional Review Board of Taipei City Psychiatric Center of Taipei City Hospital (IRB No: TCHIRB-1030408), with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of Taipei City Psychiatric Center of Taipei City Hospital. The study was conducted in Taipei City Psychiatric Center of Taipei City Hospital after obtaining the approval of its Institutional Review Board. Treatment-seeking KD patients who voluntarily admitted to an inpatient ward from January 2014 to December 2015 were consecutively screened for the eligibility of enrollment in this study. The inclusion criteria were as follows: (1) age between 18 and 60 years; (2) fulfilling DSM-IV-TR criteria for ketamine dependence as verified by two board-certified psychiatrists; (3) last ketamine use within 24 h prior to admission, validated by self-report and positive in urine toxicology test for ketamine; (4) an ability to read Chinese and provide informed consent. The exclusion criteria were: (1) other substance use disorder (including abuse and dependence) in the past year except nicotine; (2) history of schizophrenia, bipolar disorder, or major depressive disorder, or having been treated with antipsychotics, mood stabilizers (including lithium, valproic acid, carbamazepine, and quetiapine), or antidepressants; (3) history of systemic medical illnesses such as hypertension, metabolic disorders (e.g., diabetes mellitus), or renal or liver diseases; (4) history of head injury, loss of consciousness, or neurological disorders; (5) inability or refusal to provide urine sample. Healthy controls were enrolled from the physical check-up unit in the hospital with inclusion criteria as (1) age between 18 and 60 years; (2) no other substance use disorder (including abuse and dependence) in the past year, except nicotine; (3) no history of a major psychiatric disorder (including schizophrenia, schizoaffective disorder, bipolar disorder, major depressive disorder, or organic mental disorders) screened using the Mini-International Neuropsychiatric Interview (MINI) (29) by a trained psychologist; (4) no known systemic or neurological

diseases such as hypertension, metabolic disorders (e.g., diabetes mellitus), or renal or liver diseases; (5) an ability to read Chinese and provide informed consent. All the participants were given a comprehensive description of the study and then recruited after giving written informed consent.

The ward provides inpatient services in a controlled environment for individuals with substance use disorders and conducts various treatment programs, such as individual psychotherapy, group psychotherapy, family counseling, and vocational training, to help patients manage withdrawal symptoms, achieve initial abstinence, and prevent relapse. Every patient received regular drug testing to ensure their status of abstinence. Oral diazepam 20 mg/day were given on the first day of admission, and then gradually tapered off before day 5 for the treatment of withdrawal symptoms, such as anxiety, shaking, sweating, palpitations, and sleep impairment, as has been suggested previously (30–32).

Clinical Assessment

The patients underwent the following assessments in the next morning of admission after the blood sample collection was performed.

1. Ketamine use variables: The severity of ketamine dependence was measured by the Severity of Dependence Scale (SDS), which has been validated previously for the Chinese version (33). A trained research assistant was responsible for collecting the data of socio-demographic characteristics and ketamine use pattern in the past month using Time-line follow-back (TLFB). Average and maximum daily dose of ketamine as well as ketamine using days in the 30 days prior to the survey were recorded.
2. The severity of craving was measured by the Visual Analog Scale (VAS), on which the participants self-rated their level of craving for ketamine use, following a careful explanation from research assistants, by indicating a position along a continuous line between 0 and 100 mm, 0 being no craving and 100 mm being so severe that the subject was unable to resist ketamine if it is available.
3. The severity of depressive and anxiety symptoms over the past 2 weeks was assessed by the Chinese-version of the 21-item Becker Depression Inventory (BDI) (34) and the Chinese-version of the 21-item Becker Anxiety Inventory (BAI) (35).
4. Five categories of childhood trauma, including emotional, physical, and sexual abuse, and emotional and physical neglect, were assessed using the Childhood Trauma Questionnaire-short form (CTQ-SF) (36), with each subscale measured in five items and rated on a five-point Likert scale. Participants with scores exceeding the cutoff point for moderate exposure on each subscale (emotional abuse: $\geq$13; physical abuse: $\geq$10; sexual abuse: $\geq$8; emotional neglect: $\geq$15; physical neglect: $\geq$10) were classified as positive for a history of childhood trauma exposure in that category. The CTQ-SF demonstrates good internal consistency and criterion-related validity. The Chinese-version of CTQ-SF has also been validated and shown favorable factor structure and test-retest reliability (37).

Laboratory Assays

Blood sample was collected once in control participants and 3 times in KD patients on the day following admission and after 1 and 2 weeks of ketamine abstinence. The instructions about the process were given on admission and repeated on the evening prior to the day of blood withdrawal. Venous blood samples were collected and placed in ice-cold vacutainer tubes containing EDTA (1 mg/ml of blood) anti-coagulant at 8:00–9:00 a.m. after an overnight fasting. The plasma was separated by centrifugation at 3,000 $\times$ g for 15 min at 4°C, and aliquots of plasma were immediately frozen and stored at −80°C until assayed. The range of storage time for plasma samples was from 1 to 1.5 years.

Plasma oxytocin levels were determined using enzyme immunosorbent assay (Catalog number: EKE-051-01, Phoenix Pharmaceuticals, Inc., Burlingame, California, USA). Each plasma sample was assayed in duplicates and the mean of the two measurements was used for analysis. The intra-essay and inter-assay coefficients of variation was 9 and 12%, respectively. The detection range of oxytocin assay were 0–100 ng/ml (provided by the manufacturer). There was no significant cross-reactivity or interference between oxytocin and the analogs observed.

Statistical Analyses

In descriptive statistics, we used Chi-square test for categorical variables and independent *t*-test for numerical to compare demographic variables between KD and control groups.

To examine whether oxytocin differ between healthy controls and KD groups by time, we first performed a two-way ANOVA to compare mean differences among groups, adjusting for age and gender. Here, we assumed oxytocin level with no changes in week 1 and week 2 from baseline for controls.

$$y_{ijk} = \mu + \tau_i + \beta_j + \epsilon_{ijk} \begin{cases} i = 1, 2 \\ j = 1, 2, 3 \\ k = 1, \ldots, 195 \end{cases}$$

μ: overall mean effect

τ_i: the effect of case and control $\begin{cases} case \\ control \end{cases}$

β_j: the effect of the j^{th} level of Time $\begin{cases} baseline \\ week1 \\ week2 \end{cases}$

ϵ_{ijk}: is a random error component.

Pairwise *t*-test was used to test the alterations of oxytocin levels after 1 or 2 weeks of ketamine discontinuation in the KD group. Bivariate correlation analysis was used to estimate the correlations between the clinical variables, such as ketamine use variables, BDI and BAI scores, and total scores or number of types of childhood trauma assessed by CTQ-SF, and laboratory measures. SAS 9.4 (SAS Institute, Cary, NC, USA) was used for all the analyses. Significance level was set at $P < 0.05$.

RESULTS

Sample Characteristics

A total of 130 participants, including 65 KD patients and 65 age- and gender-matched controls were enrolled. The

TABLE 1 | Sociodemographic and clinical characteristics in treatment-seeking ketamine-dependent (KD) patients and controls.

	Controls (*N* = 65)	KD patients (*N* = 65)	*P*-value
Age, years, mean ± SD	31.3 ± 9.6	30.9 ± 5.7	0.77
Sex, *N* (%)			0.70
Male	44 (67.7%)	46 (70.8%)	
Female	21 (32.3%)	19 (29.2%)	
Current smoker, N (%)	5 (7.7%)	57 (95.0%)	<0.001
Ketamine use variables, mean ± SD			
Total years of ketamine use		7.9 ± 4.3	
Average daily dose in past 30 days (g/day)		3.5 ± 2.7	
Maximum daily dose in past 30 days (g/day)		7.2 ± 6.1	
Using days in past 30 days (days)		24.3 ± 10.9	
VAS for craving (0-100), mean ± SD		42.4 ± 35.2	
SDS scores, mean ± SD		10.2 ± 3.6	
BDI scores, mean ± SD		27.0 ± 12.8	
BAI scores, mean ± SD		16.7 ± 13.0	
CTQ-SF			
Number of trauma types		1.9 ± 1.5	
Total scores		61.2 ± 15.0	

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; CTQ-SF, Childhood Trauma Questionnaire-short form; SDS, Severity of Dependence Scale; VAS, Visual Analog Scale for craving.

demographic and clinical characteristics are shown in **Table 1.** The proportion of tobacco smokers was significantly higher in KD group than control group ($P < 0.001$). All of KD patients had administered ketamine for an extended period of time (7.9 ± 4.3 years), with relatively high doses (average and maximum daily dose: 3.5 ± 2.7 and 7.2 ± 6.1 g, respectively) and high frequency of use (24.3 ± 10.9 days) in the past 1 month. All of them reported the main route of ingestion as snorting without injection. The demographic and clinical assessment data were comparable between those receiving blood withdrawal at week 1 ($N = 38$) and those who did not ($N = 27$).

The Oxytocin Levels in KD Patients

Two-way ANOVA showed that oxytocin level was significantly lower in KD compared to control group. We found no significant difference of oxytocin level by time within KD groups (shown in **Figure 1**).

In pairwise *t*-tests, we found that KD patients had significantly lower levels of oxytocin at baseline compared to controls (5.89 ± 2.13 vs. 9.53 ± 4.17 ng/mL, $P < 0.001$). Thirty-eight out of 65 patients with KD provided blood samples after 1 week of ketamine abstinence whereas among them 32 patients provided blood after 2 weeks' abstinence. We found oxytocin levels increased after one (6.74 ± 2.63, $P < 0.002$) and 2 weeks (6.89 ± 2.69, $P = 0.01$) following ketamine discontinuation comparing with baseline oxytocin level; however, the oxytocin in week 1 and week 2 among KD patients were still significantly lower than controls ($P = 0.001$ and 0.002, respectively).

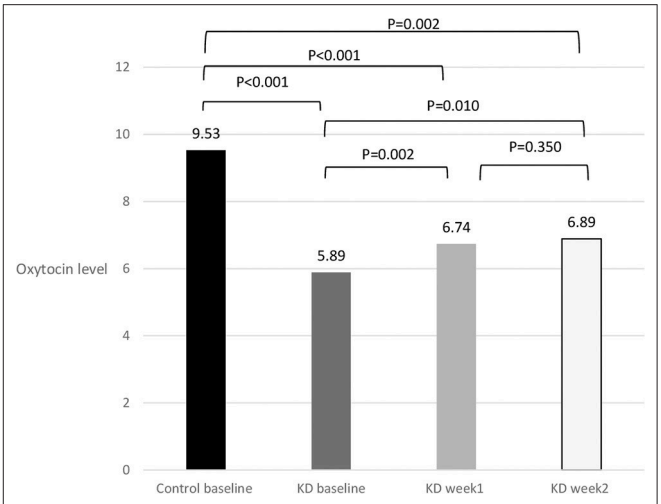

FIGURE 1 | Decreased plasma levels of oxytocin (ng/mL) following 1 and 2 weeks of ketamine discontinuation in ketamine-dependent (KD) patients.

The Correlation of Oxytocin Levels With Ketamine Use Variables, Depression or Anxiety Symptoms, and Childhood Trauma Exposure

The baseline oxytocin levels were not significantly correlated with ketamine use variables, including total years of ketamine use, daily dose and using days in the past 1 month, or VAS of ketamine craving, depression severity, and total scores or number of types of childhood trauma in CTQ-SF (*P*-values: all >0.05) (shown in **Table 2**). There was a marginal significant correlation between oxytocin levels and SDS scores ($r = -0.253$; $P = 0.058$), suggesting a trend of lower oxytocin levels in patients with higher dependence severity. Noteworthily, anxiety symptoms were negatively correlated with oxytocin levels at baseline ($r = -0.263$; $P = 0.039$).

DISCUSSION

To our knowledge, no studies to date have explored the possible association of oxytocin with chronic NMDA antagonism. Specifically, we found a significantly decreased blood level of oxytocin in patients with ketamine dependence compared to healthy controls. More interestingly, the reduction of oxytocin did not normalize after 2 weeks of withdrawal. Of note, patients with greater anxiety severity were associated with a lower oxytocin level in blood. Our preliminary findings highlight a possibility that chronic ketamine exposure is associated with a down-regulation of oxytocin and such abnormal oxytocin regulation was persistent in early abstinence.

Existing evidence indicates that oxytocin system interferes with different stages of addictive behavior across the addiction cycle (18) by reducing drug consumption, tolerance, and

TABLE 2 | The correlation of oxytocin levels at baseline with clinical variables in KD patients.

	r	*P*-value
Ketamine use variables		
Total years of ketamine use	−0.209	0.110
Average daily dose in past 30 days (g/day)	0.081	0.532
Maximum daily dose in past 30 days (g/day)	0.023	0.861
Using days in past 30 days (days)	0.064	0.621
VAS	−0.055	0.673
SDS	−0.253	0.058
BDI	−0.159	0.217
BAI	**−0.263**	**0.039**
CTQ-SF	0.013	0.924
Total scores	0.013	0.924
Number of trauma types	−0.025	0.848

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; CTQ-SF, Childhood Trauma Questionnaire-short form; SDS, Severity of Dependence Scale; VAS, Visual Analog Scale for craving. Bold highlights statistically significant values ($p < 0.05$).

reinforcing effects, suppressing both acute and protracted withdrawal, and inhibiting relapse (9–16). The possible counteracting effect of oxytocin toward addictive behaviors has also been demonstrated in recent clinical studies that proposed oxytocin might serve as a novel treatment for drug abuse (38). Intranasal oxytocin administration has been shown to be beneficial in treating individuals with alcohol (39), cannabis (40), and cocaine use disorders (41), particularly through the effect of craving reduction. Therefore, it is likely that chronic drug abuse causes a neuroadaptaional down-regulation of oxytocin, and administration of oxytocin may compensate the abnormalities and further modulate the neurobehavioral response (7, 18). In light of this point, our results also support a potential role of oxytocin in the mechanisms underlying the development of ketamine addiction. Previous studies showed that NMDA receptors are involved in the release and secretion of oxytocin (22–24). Whilst NMDA receptor activation may enhance oxytocin release, NMDA antagonist treatment has been shown to suppress the release (42) and decrease the blood level of oxytocin (24). In agreement, our patients using ketamine, an NMDA antagonist, chronically similarly displayed a reduced blood level of oxytocin.

We found oxytocin levels in KD patients increased significantly after 1 week of ketamine discontinuation despite the levels were still deficient even after 2 weeks of withdrawal compared to controls. The alterations of oxytocinergic system have been suggested to contribute to withdrawal symptoms of various substances of abuse. For example, Zanos et al. found oxytocin involves the emotional impairment of morphine abstinence in mice and oxytocin administration attenuated the negative emotional consequences (16). Similarly, oxytocin administration was reported to decrease the alcohol withdrawal-induced convulsion (19), somatic symptoms of nicotine withdrawal (20), and morphine withdrawal-related hypothermia and body weight loss (9). In line with the potential advantage of oxytocin in blocking withdrawal, some clinical evidence also showed that in alcohol-dependent individuals undergoing alcohol detoxification, oxytocin treatment was associated with a superior effect over placebo on alcohol withdrawal symptoms blockade and less symptom-triggered benzodiazepine doses needed for withdrawal management (21). Although we did not measure the severity of withdrawal sequentially, it is possible that the clinical improvement of withdrawal symptoms in KD patients was associated with an elevation of oxytocin during withdrawal. Whether there would be normalization after sustained abstinence should be examined in future studies.

In our study, patients with greater anxiety appeared to have lower oxytocin levels. This finding is in line with the current literature revealing that oxytocin is a profound anxiolytic and anti-stress factor of the brain (43). Behavioral data from the oxytocin gene-deleted mice displayed more anxiety-related behaviors and higher stress hormone (corticosterone) secretion after a stressor challenge compared to their wild-type counterparts (44). It is likely that the reduction in oxytocin may contribute to an increased level of anxiety in patients using ketamine chronically. In agreement with this notion, previous evidence demonstrated that oxytocin treatment reduces the anxiety response in rats during cocaine withdrawal (45). However, one recent study found in heroin users the plasma oxytocin levels were instead positively correlated with anxiety scores during acute withdrawal (46). The reasons for the contradictory findings are not clear. Oxytocin is thought to regulate the homeostasis of stress response mechanisms of hypothalamus-pituitary-adrenal (HPA) axis, which becomes dysfunctional following chronic drug abuse and in turn leads to an increased level of anxiety. The interactions with HPA axis are likely to have a critical role in oxytocin's effect to attenuate heightened anxiety during drug withdrawal (16, 18). Moreover, some evidence showed that different classes of drugs of abuse exhibited dissimilar effects on HPA axis activity at doses that reinforce addictive behaviors (47). For example, while ketamine may inhibit the HPA axis activity (47), chronic alcohol consumption produces a potent activation of the HPA axis in humans (48) and active heroin users showed a normalized HPA axis response (49). Given the interplay between oxytocin system and HPA axis, it is likely the expression pattern of oxytocin might differ between various drugs of abuse.

Findings from this study should be interpreted in light of several limitations. First, we only examined the oxytocin following 2 weeks of ketamine withdrawal and did not follow the depressive or anxiety symptoms after baseline. Given the persistent nature of addiction, future studies are required to examine the alterations oxytocin after a longer term of abstinence as well as how it contributes to the emotional disturbances that are commonly associated with addiction. Second, compared to patients with KD, only a small proportion of our controls were smokers and as result the differences of the biomarker levels between groups might be biased. Despite that preclinical studies suggest nicotine can modulate the oxytocin signaling (20, 50), no human studies to date have reported the association of oxytocin with tobacco smoking. In addition, we did not find a significant correlation between oxytocin levels and smoking amount in

patients with KD ($P = 0.22$). Therefore, the effect of tobacco smoking on oxytocin levels might be limited. Third, using plasma oxytocin as a surrogate for central oxytocin function is still controversial because peripheral concentrations may also be contributed by peripheral organs including the gastrointestinal tract, heart, and reproductive tract (51). Despite an affirmative conclusion is still lacking, one recent meta-analysis showed a positive association may exist between central and peripheral oxytocin concentrations (52). Last, apart from the effect of chronic ketamine administration on the oxytocin expression, other individual factors may also contribute to the differences in oxytocin system, such as genetic variation, early life adversities, or stressful social experiences, and thus confound the results (53). We were unable to confirm whether the oxytocin expression in KD patients had been different from normal controls before they ingested ketamine, or a causal relationship between heavy ketamine use and deficient oxytocin expression.

In summary, we found that oxytocin levels were distinctively reduced in KD patients compared to controls and the reduction was persistent throughout the first 2 weeks of baseline was observed. In addition, a lower level of oxytocin was associated with greater anxiety symptoms. These results suggest that oxytocin down-regulation may contribute to the neuroadaptational mechanisms of ketamine addiction and a lower oxytocin might be associated with the anxious phenotype of ketamine addiction. Our data may further provide insight into the neurochemical changes of dysregulated oxytocin signaling after chronic NMDA antagonism. Whether there will be a delayed normalization of oxytocin expression after a longer-term of abstinence should be further testified. Given the growing interests in the utility of oxytocin-based therapeutic approaches to drug and alcohol addiction (7, 18), it might be worth examining the relevance of oxyctocin administration to ketamine dependence treatment in future studies.

AUTHOR CONTRIBUTIONS

M-CH designed the study and wrote the manuscript. L-YC and H-MC recruited participants and assisted in data interpretation. X-YL and W-JC did the statistical analysis and drafted the results section. C-KC assisted with the design of the analysis and provided statistical consultancy. KX revised the final version. W-JC incorporated edits from co-authors and was responsible for communication.

FUNDING

This study was supported by grants from Ministry of Science and Technology, Taiwan (103-2628-B-532-001-MY3 and 106-2314-B-532-005-MY3), Chang Gung Memorial Hospital, Taiwan (CMRPG2D0261, CMRPG8C1291, and CMRPG8D0481), Taipei City Hospital, Taiwan (10701-62-029 and TPCH-106-055), and National Center for Post-Traumatic Stress Disorder, United States.

REFERENCES

1. Kalsi SS, Wood DM, Dargan PI. The epidemiology and patterns of acute and chronic toxicity associated with recreational ketamine use. *Emerg Health Threats J*. (2011) 4:7107. doi: 10.3402/ehtj.v4i0.7107
2. Li JH, Vicknasingam B, Cheung YW, Zhou W, Nurhidayat AW, Jarlais DC, et al. To use or not to use: an update on licit and illicit ketamine use. *Subst Abuse Rehabil*. (2011) 2:11–20. doi: 10.2147/SAR.S15458
3. Liao Y, Tang YL, Hao W. Ketamine and international regulations. *Am J Drug Alcohol Abuse* (2017) 43:495–504. doi: 10.1080/00952990.2016.1278449
4. Vidal Gine C, Fernandez Calderon F, Lopez Guerrero J. Patterns of use, harm reduction strategies, and their relation to risk behavior and harm in recreational ketamine users. *Am J Drug Alcohol Abuse* (2016) 42:358–69. doi: 10.3109/00952990.2016.1141211
5. Feng LY, Yu WJ, Chang WT, Han E, Chung H, Li JH. Comparison of illegal drug use pattern in Taiwan and Korea from 2006 to 2014. *Subst Abuse Treat Prev Policy* (2016) 11:34. doi: 10.1186/s13011-016-0078-x
6. Huang MC, Chen LY, Chen CK, Lin SK. Potential benefit of lamotrigine in managing ketamine use disorder. *Med Hypotheses* (2016) 87:97–100. doi: 10.1016/j.mehy.2015.11.011
7. Lee MR, Weerts EM. Oxytocin for the treatment of drug and alcohol use disorders. *Behav Pharmacol*. (2016) 27:640–8. doi: 10.1097/FBP.0000000000000258
8. Olff M, Langeland W, Witteveen A, Denys D. A psychobiological rationale for oxytocin in the treatment of posttraumatic stress disorder. *CNS Spectr*. (2010) 15:522–30. doi: 10.1017/S109285290000047X
9. Kovacs GL, Sarnyai Z, Szabo G. Oxytocin and addiction: a review. *Psychoneuroendocrinology* (1998) 23:945–62. doi: 10.1016/S0306-4530(98)00064-X
10. Szabo G, Kovacs GL, Telegdy G. Intraventricular administration of neurohypophyseal hormones interferes with the development of tolerance to ethanol. *Acta Physiol Hung*. (1989) 73:97–103.
11. King CE, Griffin WC, Luderman LN, Kates MM, McGinty JF, Becker HC. Oxytocin Reduces Ethanol Self-Administration in Mice. *Alcohol Clin Exp Res*. (2017) 41:955–64. doi: 10.1111/acer.13359
12. Qi J, Yang JY, Wang F, Zhao YN, Song M, Wu CF. Effects of oxytocin on methamphetamine-induced conditioned place preference and the possible role of glutamatergic neurotransmission in the medial prefrontal cortex of mice in reinstatement. *Neuropharmacology* (2009) 56:856–65. doi: 10.1016/j.neuropharm.2009.01.010
13. Zhou L, Sun WL, Young AB, Lee K, McGinty JF, See RE. Oxytocin reduces cocaine seeking and reverses chronic cocaine-induced changes in glutamate receptor function. *Int J Neuropsychopharmacol*. (2014) 18: pyu009. doi: 10.1093/ijnp/pyu009
14. Bahi A. The oxytocin receptor impairs ethanol reward in mice. *Physiol Behav*. (2015) 139:321–7. doi: 10.1016/j.physbeh.2014.11.046
15. Han WY, Du P, Fu SY, Wang F, Song M, Wu CF, et al. Oxytocin via its receptor affects restraint stress-induced methamphetamine CPP reinstatement in mice: involvement of the medial prefrontal cortex and dorsal hippocampus glutamatergic system. *Pharmacol Biochem Behav*. (2014) 119:80–7. doi: 10.1016/j.pbb.2013.11.014
16. Zanos P, Georgiou P, Wright SR, Hourani SM, Kitchen I, Winsky-Sommerer R, et al. The oxytocin analogue carbetocin prevents emotional impairment and stress-induced reinstatement of opioid-seeking in morphine-abstinent mice. *Neuropsychopharmacology* (2014) 39:855–65. doi: 10.1038/npp.2013.285
17. Lin SH, Lee LT, Tsai HC, Chen KC, Chen WT, Lee IH, et al. Association between blood level of plasma oxytocin and novelty seeking among methadone-maintained heroin users. *Neuropsychobiology* (2015) 71:65–9. doi: 10.1159/000371637
18. Bowen MT, Neumann ID. Rebalancing the addicted brain: oxytocin interference with the neural substrates of addiction. *Trends Neurosci*. (2017) 40:691–708. doi: 10.1016/j.tins.2017.10.003
19. Szabo G, Kovacs GL, Telegdy G. Effects of neurohypophyseal peptide hormones on alcohol dependence and withdrawal.

Alcohol Alcohol (1987) 22:71–4. doi: 10.1093/oxfordjournals.alcalc.a044668
20. Manbeck KE, Shelley D, Schmidt CE, Harris AC. Effects of oxytocin on nicotine withdrawal in rats. *Pharmacol Biochem Behav*. (2014) 116:84–9. doi: 10.1016/j.pbb.2013.11.002
21. Pedersen CA, Smedley KL, Leserman J, Jarskog LF, Rau SW, Kampov-Polevoi A, et al. Intranasal oxytocin blocks alcohol withdrawal in human subjects. *Alcohol Clin Exp Res*. (2013) 37:484–9. doi: 10.1111/j.1530-0277.2012.01958.x
22. Herman JP, Eyigor O, Ziegler DR, Jennes L. Expression of ionotropic glutamate receptor subunit mRNAs in the hypothalamic paraventricular nucleus of the rat. *J Comp Neurol*. (2000) 422:352–62. doi: 10.1002/1096-9861(20000703)422:3<352::AID-CNE3>3.0.CO;2-F
23. van den Pol AN, Wuarin JP, Dudek FE. Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. *Science* (1990) 250:1276–8. doi: 10.1126/science.1978759
24. Onaka T, Yagi K. Involvement of N-methyl-D-aspartic acid receptor activation in oxytocin and vasopressin release after osmotic stimuli in rats. *J Neuroendocrinol*. (2001) 13:166–74. doi: 10.1111/j.1365-2826.2001.00607.x
25. Yagi K, Onaka T, Yoshida A. Role of NMDA receptors in the emotional memory associated with neuroendocrine responses to conditioned fear stimuli in the rat. *Neurosci Res*. (1998) 30:279–86.
26. Zimmermann FF, Gaspary KV, Siebel AM, Bonan CD. Oxytocin reversed MK-801-induced social interaction and aggression deficits in zebrafish. *Behav Brain Res*. (2016) 311:368–74. doi: 10.1016/j.bbr.2016.05.059
27. Mizuki R, Fujiwara T. Association of oxytocin level and less severe forms of childhood maltreatment history among healthy Japanese adults involved with child care. *Front Behav Neurosci*. (2015) 9:138. doi: 10.3389/fnbeh.2015.00138
28. Scantamburlo G, Hansenne M, Fuchs S, Pitchot W, Marechal P, Pequeux C, et al. Plasma oxytocin levels and anxiety in patients with major depression. *Psychoneuroendocrinology* (2007) 32:407–10. doi: 10.1016/j.psyneuen.2007.01.009
29. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* (1998) 59(Suppl. 20):22–33;quiz 4-57.
30. Critchlow DG. A case of ketamine dependence with discontinuation symptoms. *Addiction* (2006) 101:1212–3. doi: 10.1111/j.1360-0443.2006.01494.x
31. Morgan CJ, Curran HV, Independent Scientific Committee on D. Ketamine use: a review. *Addiction* (2012) 107:27–38. doi: 10.1111/j.1360-0443.2011.03576.x
32. Chen WY, Huang MC, Lin SK. Gender differences in subjective discontinuation symptoms associated with ketamine use. *Subst Abuse Treat Prev Policy* (2014) 9:39. doi: 10.1186/1747-597X-9-39
33. Chen VC, Chen H, Lin TY, Chou HH, Lai TJ, Ferri CP, et al. Severity of heroin dependence in Taiwan: reliability and validity of the Chinese version of the Severity of Dependence Scale (SDS[Ch]). *Addict Behav*. (2008) 33:1590–3. doi: 10.1016/j.addbeh.2008.06.001
34. Lu ML, Che HH, Chang SW, Shen W. Reliability and validity of the Chinese version of the beck depression inventory-II. *Taiwanes J Psychiatry* (2002) 16:301–10. doi: 10.1016/j.yebeh.2015.01.019
35. Che HH, Lu ML, Chen HC, Chang SW, Lee YJ. 10.6320/FJM.2006.10. *Formosan J Med*. (2006) 10:447–54. doi: 10.6320/FJM.2006.10
36. Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, et al. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse Negl*. (2003) 27:169–90. doi: 10.1016/S0145-2134(02)00541-0
37. Cheng YC, Chen CH, Chou KR, Kuo PH, Huang MC. Reliability and factor structure of the chinese version of childhood trauma questionnaire-short form in patients with substance use disorder. *Taiwanese J Psychiatry* (2018) 32:52–62. doi: 10.29478/TJP
38. McGregor IS, Bowen MT. Breaking the loop: oxytocin as a potential treatment for drug addiction. *Horm Behav*. (2012) 61:331–9. doi: 10.1016/j.yhbeh.2011.12.001
39. Mitchell JM, Arcuni PA, Weinstein D, Woolley JD. Intranasal oxytocin selectively modulates social perception, craving, and approach behavior in subjects with alcohol use disorder. *J Addict Med*. (2016) 10:182–9. doi: 10.1097/ADM.0000000000000213
40. McRae-Clark AL, Baker NL, Maria MM, Brady KT. Effect of oxytocin on craving and stress response in marijuana-dependent individuals: a pilot study. *Psychopharmacology* (2013) 228:623–31. doi: 10.1007/s00213-013-3062-4
41. Lee MR, Glassman M, King C, B., Kelly DL, Stein EA, Schroeder J, et al. Complexity of oxytocin's effects in a chronic cocaine dependent population. *Eur Neuropsychopharmacol*. (2014) 24:1483–91. doi: 10.1016/j.euroneuro.2014.06.005
42. Parker SL, Crowley WR. Central stimulation of oxytocin release in the lactating rat by N-methyl-D-aspartate: requirement for coactivation through non-NMDA glutamate receptors or the glycine coagonist site. *Neuroendocrinology* (1995) 62:467–78. doi: 10.1159/000127036
43. Neumann ID, Slattery DA. Oxytocin in general anxiety and social fear: a translational approach. *Biol Psychiatry* (2016) 79:213–21. doi: 10.1016/j.biopsych.2015.06.004
44. Amico JA, Mantella RC, Vollmer RR, Li X. Anxiety and stress responses in female oxytocin deficient mice. *J Neuroendocrinol*. (2004) 16:319–24. doi: 10.1111/j.0953-8194.2004.01161.x
45. Morales-Rivera A, Hernandez-Burgos MM, Martinez-Rivera A, Perez-Colon J, Rivera R, Montalvo J, et al. Anxiolytic effects of oxytocin in cue-induced cocaine seeking behavior in rats. *Psychopharmacology* (2014) 231:4145–55. doi: 10.1007/s00213-014-3553-y
46. Nikolaou K, Kapoukranidou D, Ndungu S, Floros G, Kovatsi L. Severity of withdrawal symptoms, plasma oxytocin levels, and treatment outcome in heroin users undergoing acute withdrawal. *J Psychoactive Drugs* (2017) 49:233–41. doi: 10.1080/02791072.2017.1312644
47. Broadbear JH, Winger G, Woods JH. Self-administration of fentanyl, cocaine and ketamine: effects on the pituitary-adrenal axis in rhesus monkeys. *Psychopharmacology* (2004) 176:398–406. doi: 10.1007/s00213-004-1891-x
48. Zhou Y, Kreek MJ. Alcohol: a stimulant activating brain stress responsive systems with persistent neuroadaptation. *Neuropharmacology* (2014) 87:51–8. doi: 10.1016/j.neuropharm.2014.05.044
49. Walter M, Gerber H, Kuhl HC, Schmid O, Joechle W, Lanz C, et al. Acute effects of intravenous heroin on the hypothalamic-pituitary-adrenal axis response: a controlled trial. *J Clin Psychopharmacol*. (2013) 33:193–8. doi: 10.1097/JCP.0b013e31828393cb
50. Zanos P, Georgiou P, Metaxas A, Kitchen I, Winsky-Sommerer R, Bailey A. Region-specific up-regulation of oxytocin receptor binding in the brain of mice following chronic nicotine administration. *Neurosci Lett*. (2015) 600:33–7. doi: 10.1016/j.neulet.2015.05.054
51. Leng G, Ludwig M. Intranasal oxytocin: myths and delusions. *Biol Psychiatry* (2016) 79:243–50. doi: 10.1016/j.biopsych.2015.05.003
52. Valstad M, Alvares GA, Egknud M, Matziorinis AM, Andreassen OA, Westlye LT, et al. The correlation between central and peripheral oxytocin concentrations: a systematic review and meta-analysis. *Neurosci Biobehav Rev*. (2017) 78:117–24. doi: 10.1016/j.neubiorev.2017.04.017
53. Buisman-Pijlman FT, Sumracki NM, Gordon JJ, Hull PR, Carter CS, Tops M. Individual differences underlying susceptibility to addiction: Role for the endogenous oxytocin system. *Pharmacol Biochem Behav*. (2014) 119:22–38. doi: 10.1016/j.pbb.2013.09.005

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 19 December 2018
doi: 10.3389/fpsyt.2018.00713

Blood Levels of Glutamate and Glutamine in Recent Onset and Chronic Schizophrenia

Caroline Madeira[1], Flavio V. Alheira[2], Marilia A. Calcia[2], Thuany C. S. Silva[2], Filippe M. Tannos[2], Charles Vargas-Lopes[1], Melissa Fisher[3,4], Nelson Goldenstein[2], Marco Antonio Brasil[2], Sophia Vinogradov[3,4], Sergio T. Ferreira[5,6] and Rogerio Panizzutti[1,2]**

[1] Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2] Serviço de Psiquiatria e Psicologia Médica, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [3] Department of Psychiatry, School of Medicine, University of California, San Francisco, San Francisco, CA, United States, [4] Department of Psychiatry, University of Minnesota, Minneapolis, MN, United States, [5] Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [6] Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

OPEN ACCESS

Edited by:
Hsien-Yuan Lane,
China Medical University, Taiwan

Reviewed by:
Svetlana Ivanova,
Mental Health Research Institute, Tomsk NRMC, Russia
Stefania Schiavone,
University of Foggia, Italy

***Correspondence:**
Sergio T. Ferreira
ferreira@bioqmed.ufrj.br
Rogerio Panizzutti
rogerio@icb.ufrj.br

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *30 September 2018*
Accepted: *05 December 2018*
Published: *19 December 2018*

Citation:
Madeira C, Alheira FV, Calcia MA, Silva TCS, Tannos FM, Vargas-Lopes C, Fisher M, Goldenstein N, Brasil MA, Vinogradov S, Ferreira ST and Panizzutti R (2018) Blood Levels of Glutamate and Glutamine in Recent Onset and Chronic Schizophrenia. Front. Psychiatry 9:713. doi: 10.3389/fpsyt.2018.00713

Converging evidence indicates that dysfunctions in glutamatergic neurotransmission and in the glutamate-glutamine cycle play a role in the pathophysiology of schizophrenia. Here, we investigated glutamate and glutamine levels in the blood of patients with recent onset schizophrenia or chronic schizophrenia compared to healthy controls. Compared with healthy controls, patients with recent onset schizophrenia showed increased glutamine/glutamate ratio, while patients with chronic schizophrenia showed decreased glutamine/glutamate ratio. Results indicate that circulating glutamate and glutamine levels exhibit a dual behavior in schizophrenia, with an increase of glutamine/glutamate ratio at the onset of schizophrenia followed by a decrease with progression of the disorder. Further studies are warranted to elucidate the mechanisms and consequences of changes in circulating glutamate and glutamine in schizophrenia.

Keywords: recent onset schizophrenia, chronic schizophrenia, glutamate, glutamine, blood

INTRODUCTION

Schizophrenia is a heterogeneous disorder characterized by a wide range of symptoms, including positive (hallucinations, delusions, disorganized thinking) and negative symptoms (affective flattening, alogia, apathy), and cognitive impairments (in memory and executive functions) (1, 2). Considerable evidence indicates that dysfunctional glutamatergic neurotransmission is involved in the pathophysiology of schizophrenia (3, 4). Among different glutamate receptor subtypes, hypofunction of N-methyl-D-aspartate (NMDA) receptors has been particularly implicated in schizophrenia. This comes from observations that administration of the NMDA receptor antagonists, phencyclidine (PCP) and ketamine, induces schizophrenia-like symptomatology in healthy individuals and exacerbates symptoms in patients with schizophrenia (5–7). Additionally, dysfunctions in metabotropic glutamate receptors (mGluRs), which can modulate NMDA receptor function, have also been implicated in schizophrenia (8, 9). Increased levels of mGluR1protein and mRNA have been reported in schizophrenia patients (10, 11). mGluR2/3 agonists improved both positive and negative symptoms of schizophrenia patients (12) and reduced cognitive deficits induced by ketamine in healthy volunteers (13). Finally, a polymorphism in the mGluR7 gene was associated with schizophrenia (14).

Glutamatergic neurotransmission is initiated by the release of glutamate into the synaptic cleft, where it binds to and activates glutamate receptors at the postsynaptic terminal membrane. Glutamate is subsequently removed from the synaptic cleft by astrocytes and converted to glutamine by glutamine synthetase (15). Glutamine can then be transported into the presynaptic neuron and reconverted to glutamate by phosphate-activated glutaminase (16, 17). Abnormalities in the glutamate-glutamine cycle have been associated with schizophrenia and may contribute to the dysfunction in glutamatergic neurotransmission (18, 19). Evidence indicates that glutamine synthetase protein levels are decreased in schizophrenia (19, 20). On the other hand, other studies found increased expression and enzymatic activity of the phosphate-activated glutaminase in schizophrenia (18, 21).

Changes in glutamate levels in the central nervous system (CNS) may reflect changes in blood levels and vice-versa, since a positive correlation has been reported between glutamate levels in the blood and in the CNS (22, 23). Altered glutamate and glutamine levels in the blood have been reported in schizophrenia, but results are not consistent. For example, previous studies have found increased blood levels of glutamate in patients with chronic schizophrenia compared with healthy controls (24, 25), but no difference was observed in acute schizophrenia (26). Interestingly, one study reported decreased blood glutamate levels in first episode psychosis (27). Blood glutamine levels were reported to be decreased in patients with schizophrenia (28), although another study found no difference in blood glutamine levels of individuals with schizophrenia compared with healthy controls (26).

The above findings indicate that changes in peripheral glutamate and glutamine may occur in schizophrenia, but the direction of change appears to depend on the duration of the disorder. We now report measurements of glutamate and glutamine levels in the blood of patients with recent onset schizophrenia or chronic schizophrenia compared to healthy controls.

MATERIALS AND METHODS

Participants

Thirty-two patients with recent onset (within 5 years of illness onset) and 56 patients with chronic schizophrenia were recruited from community mental health centers and outpatient clinics in the Bay Area of San Francisco, USA, to participate in randomized controlled trials of computerized cognitive training (ClinicalTrials.gov NCT00312962 and NCT00694889) (29–31). Blood collection was performed prior to the beginning of cognitive training. Fifty-three healthy control subjects were recruited from the same local community in the USA. In Brazil, 67 patients with chronic schizophrenia were recruited at the Institute of Psychiatry of the Federal University of Rio de Janeiro (IPUB-UFRJ) and the Psychiatry Center of Rio de Janeiro (CPRJ). Seventy-five healthy control subjects were recruited from the same local community in Brazil. Ethical approval for this study was obtained from the Committee for Ethics in Human Research from each participant institution. All subjects or their legal guardian provided written informed consent for study participation.

Patients with schizophrenia were diagnosed using the Structured Clinical Interview (SCID-I) for DSM-IV and evaluated using the Brief Psychiatry Rating Scale (BPRS). We used the BPRS because it is briefer and less time consuming than the Positive and Negative Syndrome Scale (PANSS), and there is a strong linear association between the two scales (32). Exclusion criteria were comorbid psychiatric diagnosis, substance abuse, intellectual disability, renal disease, epilepsy, and history of trauma or tumor in the central nervous system. Healthy controls had good general physical health and no Axis I or Axis II psychiatric disorder (SCID-NP), and no current or previous use of psychotropic medication.

Determination of Glutamate and Glutamine Levels

Available samples comprised serum from the two clinical trial cohorts in the USA and plasma from the cross-sectional study in Brazil. Importantly, we note that a recent meta-analysis showed that altered levels of glutamate are found in both plasma and serum of patients with schizophrenia (33). Blood samples from patients or healthy controls were drawn before lunch, around noon, and stored at −80°C until analysis. To extract free amino acids, trichloroacetic acid (TCA; 5% final concentration) was added to each sample, followed by centrifugation for 5 min. at 14,500 rpm at room temperature, separation of the supernatant and removal of TCA by extraction with saturated ether. Derivatization was performed with o-phtalaldehyde and N-tert-butyloxycarbonyl-L-cysteine (34). Separation and detection of derivatized amino acids was performed by high-performance liquid chromatography (34, 35) with a linear gradient of acetonitrile using a C18 reverse phase column. The investigators (CM and CVL) were blind to the group identity of the samples.

Statistical Analysis

Data are presented as means ± SD (standard deviation). The distributions of glutamate and glutamine concentrations were evaluated for missing values and normalcy. We adjusted outliers by winsorization (36) replacing them with the next value plus or minus 10% (depending on whether the outlier is positive or negative). For glutamate, two outliers were adjusted in the healthy control group and two outliers were adjusted in the recent onset schizophrenia group. For the glutamine/glutamate ratio, two outliers were adjusted in the healthy control group and two outliers were adjusted in the recent onset schizophrenia group. In the chronic schizophrenia cohort from the USA, for glutamate we adjusted one outlier in the healthy control group and two outliers in the chronic schizophrenia group. For glutamine, we adjusted three outliers in the chronic schizophrenia group. For the glutamine/glutamate ratio, we adjusted one outlier in the healthy control group and three outliers in the chronic schizophrenia group. In the chronic schizophrenia cohort from Brazil, for glutamate we adjusted three outliers in the healthy control group and four outliers in the chronic schizophrenia group. For glutamine, we adjusted one outlier in the healthy control

TABLE 1 | Characteristics of study subjects with recent onset or chronic schizophrenia from the USA cohort.

	Healthy controls	Recent onset schizophrenia	*p*	Healthy controls	Chronic schizophrenia	*p*
Sex, M/F	17/21	25/7	0.005	10/5	40/16	0.72
Age, years (range)	18.8 ± 3.8 (12–25)	21.0 ± 3.7 (15–29)	0.015	44.9 ± 11.2 (26–57)	45.9 ± 8.3 (26–59)	0.75
Illness duration, years (range)	N/A	1.82 ± 1.68 (0.08–5)	N/A	N/A	23.6 ± 10.21 (6–41)	N/A
CPZ equivalents (mg/day) (range)	N/A	243.3 ± 133.9 (25.1–562.9)	N/A	N/A	402.7 ± 330.2 (0–1918.0)	N/A
Antipsychotic in use (typical/atypical/combination/no medication)	N/A	1/24/1/5	N/A	N/A	7/42/3/2	N/A
Smoker/ Non-smoker	1/36	20/8	0.003	3/12	31/25	0.015
BPRS total score (range)	N/A	42.5 ± 9.2 (26–62)	N/A	N/A	35.2 ± 9.2 (18–56)	N/A
BPRS negative score (range)	N/A	16.5 ± 6.4 (8–36)	N/A	N/A	17.6 ± 6.3 (9–38)	N/A
BPRS positive score (range)	N/A	13.7 ± 4.8 (7–22)	N/A	N/A	18.4 ± 5.5 (8–29)	N/A

Comparisons between the two groups were performed using χ^2 test for sex and smoking status, and t-test for age. Values are shown as means ± standard deviation (range). BPRS, Brief Psychiatric Rating Scale; CPZ, chlorpromazine; N/A, not applicable. Typical antipsychotics included flufenazine, clorpromazine, amoxapine, haloperidol, perphenazine, thioridazine, and thiothixene. Atypical antipsychotics included clozapine, olanzapine, quetiapine, risperidone, ziprasidone, and aripiprazol.

TABLE 2 | Blood levels of glutamate and glutamine in recent onset schizophrenia (USA cohort) adjusted for age, sex, and smoking status as covariates.

			95% C.I.		
Amino acids	**Group**	**Mean**	**Lower bound**	**Upper bound**	**Statistics**
Glutamate, μmol/L	Healthy controls	201.5	177.3	225.8	$F = 12.6$, $p = 0.001$
	Recent onset schizophrenia	131.2	102.9	159.6	
Glutamine, μmol/L	Healthy controls	505.3	459.0	551.5	$F = 12.8$, $p = 0.001$
	Recent onset schizophrenia	370.4	316.3	424.5	
Glutamine/ glutamate ratio	Healthy controls	2.73	1.99	3.46	$F = 4.47$, $p = 0.04$
	Recent onset schizophrenia	3.99	3.14	4.85	

C.I., confidence interval.

group and two outliers in the chronic schizophrenia group. For the glutamine/glutamate ratio, we adjusted three outliers in the healthy control group and three outliers in the chronic schizophrenia group. Statistical significances of differences between groups were determined by unpaired *t*-test with Welch's correction. Differences between groups for gender and smoking status were analyzed using chi-square. Differences in glutamate or glutamine levels between healthy controls and patients with schizophrenia (recent onset or chronic) groups were tested using ANCOVA with age, sex or smoking status as covariates as appropriate.

RESULTS

Blood Glutamate and Glutamine Levels in Patients With Recent Onset Schizophrenia

We first asked whether circulating levels of glutamate and glutamine were altered in a cohort of 32 patients with recent onset schizophrenia (5 or less years of disorder) compared with 38 healthy controls. The mean age of patients with recent onset schizophrenia was 2 years older than the mean age of the healthy controls and this difference was significant at group level ($t = 2.5$, $p = 0.01$) (**Table 1**). However, no significant correlations were observed between age and blood levels of glutamate or glutamine in either healthy controls or recent onset schizophrenia groups (**Figure S1**). Sex distribution was different between groups, with the schizophrenia group including more men ($X^2 = 8.07$, $p = 0.005$). No significant difference in glutamine levels was observed between male and female healthy controls (**Figure S2**). In contrast, glutamate levels were significantly higher in males than in female patients with recent onset schizophrenia (151.7 ± 15.0 vs. 83.4 ± 16.8 μmol/L, respectively; $t = 2.28$; $p = 0.03$; **Figure S2**).

The proportion between smokers and non-smokers was also different between the two groups ($X^2 = 8.94$, $p = 0.003$). The group of patients with recent onset schizophrenia included more smokers than the healthy control group (28.6 vs. 2.7% smokers, respectively). We therefore introduced age, sex and smoking status as covariates in the analysis and found that blood glutamate and glutamine levels were ∼30% lower in patients with recent onset schizophrenia compared to healthy controls (**Table 2**). Further, the glutamine/glutamate ratio was significantly higher in patients with recent onset schizophrenia than in healthy controls (**Table 2**).

TABLE 3 | Blood levels of glutamate and glutamine in chronic schizophrenia (USA cohort) using smoking status as covariate.

Amino acids	Group	Mean	95% C.I.		Statistics
			Lower bound	Upper bound	
Glutamate, μmol/L	Healthy controls	180.3	101.1	259.5	$F = 3.5$, $p = 0.066$
	Chronic schizophrenia	254.6	221.4	287.8	
Glutamine, μmol/L	Healthy controls	472.8	377.1	568.6	$F = 19.8$, $p = 0.0001$
	Chronic schizophrenia	259.1	219.0	299.3	
Glutamine/ glutamate ratio	Healthy controls	4.13	3.25	5.02	$F = 32.0$, $p = 0.0001$
	Chronic schizophrenia	1.62	1.25	1.99	

C.I., confidence interval.

In patients with recent onset schizophrenia, glutamate and glutamine levels were not significantly correlated to total BPRS score ($r = 0.22$, $p = 0.22$ for glutamate; $r = -0.33$, $p = 0.06$ for glutamine), to the BPRS score for negative symptoms ($r = 0.19$, $p = 0.28$ for glutamate; $r = -0.21$, $p = 0.24$ for glutamine), or to the BPRS score for positive symptoms ($r = 0.16$, $p = 0.39$ for glutamate; $r = -0.27$, $p = 0.13$ for glutamine). Moreover, no significant correlation was observed between use of antipsychotic medications (expressed as chlorpromazine equivalents) and glutamate or glutamine levels ($r = -0.09$, $p = 0.66$ for glutamate; $r = 0.17$, $p = 0.43$, for glutamine; **Figure S3**).

Blood Glutamate and Glutamine Levels in Patients With Chronic Schizophrenia

Next, we asked whether patients with chronic schizophrenia showed altered glutamate and glutamine levels compared to healthy controls. Patients with chronic schizophrenia and healthy controls had similar age and sex distributions, but patients with schizophrenia were more likely to smoke ($X^2 = 5.93$, $p = 0.015$) (**Table 1**). The smoking status was thus included as a covariate in the analysis (**Table 3**). Patients with chronic schizophrenia showed a trend of increase in blood glutamate levels compared to healthy controls ($F = 3.50$; $p = 0.066$). On the other hand, glutamine levels were significantly lower in patients with chronic schizophrenia than in healthy controls ($F = 19.82$; $p = 0.0001$). As a result, the glutamine/glutamate ratio was lower in patients with chronic schizophrenia than in healthy controls ($F = 32.05$, $p = 0.0001$).

No significant correlation was observed between age and levels of glutamate or glutamine in healthy controls (**Figure S4**). In contrast, age was significantly correlated with glutamate ($r = 0.30$, $p = 0.02$) and glutamine levels ($r = -0.27$, $p = 0.04$) in patients with chronic schizophrenia (**Figure S4**).

We evaluated the correlation between blood amino acid levels and the current use of medications and found that glutamate and glutamine levels were significantly and negatively correlated to the daily use of antipsychotic medications (measured in chlorpromazine equivalents) ($r = 0.28$, $p = 0.04$ for glutamate; $r = -0.30$, $p = 0.02$, for glutamine; **Figure 1**). We further evaluated the correlation between blood glutamate and glutamine levels and the severity of symptoms in patients with chronic schizophrenia. Glutamate and glutamine levels were correlated to total BPRS score ($r = -0.35$, $p = 0.01$ for glutamate; $r = 0.26$, $p = 0.05$ for glutamine), but not to the BPRS score for positive symptoms ($r = -0.25$, $p = 0.06$ for glutamate; $r = 0.05$, $p = 0.72$ for glutamine) or to the BPRS score for negative symptoms ($r = -0.07$, $p = 0.63$ for glutamate; $r = 0.1$, $p = 0.48$ for glutamine).

Glutamate and Glutamine Levels in the Blood of Patients With Chronic Schizophrenia From Brazil

To validate and extend the above described findings (obtained in cohorts from the USA), we recruited a cohort of patients with chronic schizophrenia from Brazil and compared blood glutamate and glutamine levels with age- and sex-matched healthy controls. Although patient and control groups were quite similar in terms of age and sex, the group of patients with chronic schizophrenia included significantly more smokers than the healthy controls group ($X^2 = 13.24$, $p = 0.0001$) (**Table 4**). Thus, we included smoking status as a covariate in the analysis (**Table 5**). Results showed that patients with chronic schizophrenia had significantly increased blood glutamate levels compared to healthy controls ($F = 16.3$, $p = 0.0001$). Moreover, blood glutamine levels were significantly lower in patients with chronic schizophrenia than in healthy controls ($F = 55.6$, $p = 0.0001$). As a result, the glutamine/glutamate ratio in the blood was significantly lower in patients with chronic schizophrenia than in healthy controls ($F = 27.0$, $p = 0.0001$).

Glutamine levels were negatively correlated to age in healthy controls ($r = -0.31$, $p = 0.01$), but not in chronic schizophrenia patients ($r = 0.01$, $p = 0.94$). Glutamate levels were not correlated to age in both groups (healthy controls: $r = 0.14$, $p = 0.24$; chronic schizophrenia: $r = 0.01$, $p = 0.92$). Glutamate and glutamine levels were significantly correlated to the current use of antipsychotic medications (expressed as chlorpromazine equivalents) ($r = -0.49$, $p = 0.0001$ for glutamate; $r = 0.35$, $p = 0.02$, for glutamine; **Figure 2**). We compared levels of glutamate and glutamine in patients using typical antipsychotics vs. those using atypical antipsychotics. The glutamine/glutamate ratio was significantly lower in patients using typical antipsychotics than on those using atypical antipsychotics (0.57 vs. 1.25, $F = 4.23$, $p = 0.046$) (**Table S1**).

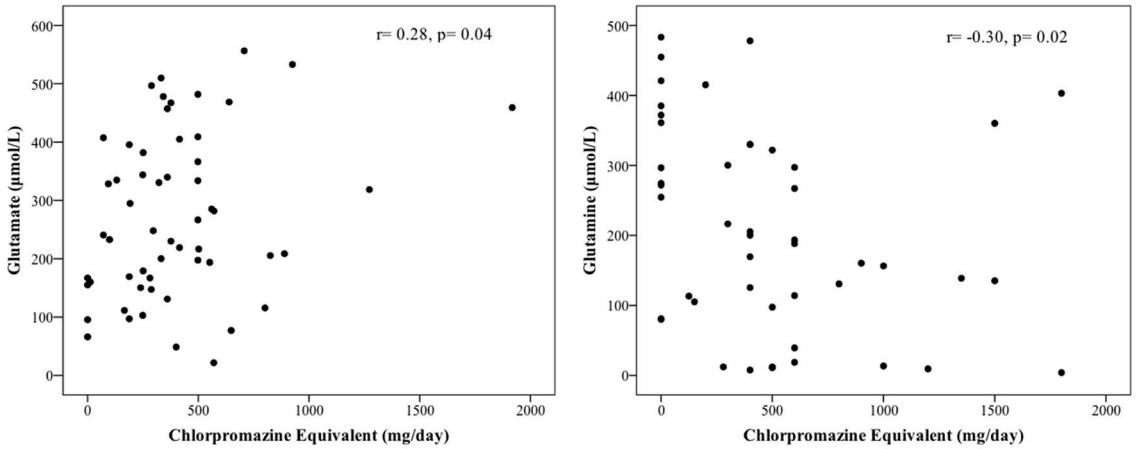

FIGURE 1 | Correlation between blood levels of glutamate and glutamine and the use of antipsychotic medication in chronic schizophrenia (USA cohort).

TABLE 4 | Characteristics of study subjects with chronic schizophrenia from the Brazilian cohort.

	Healthy controls	Chronic schizophrenia	*p*-value
Sex, M/F	34/41	27/40	0.54
Age, years (range)	39.1 ± 13.2 (20–72)	42.4 ± 10.9 (25–68)	0.11
Illness duration, years (range)	N/A	22.4 ± 11.4 (6–54)	N/A
CPZ equivalents (mg/day) (range)	N/A	104.4 ± 94.1 (0–430.2)	N/A
Antipsychotic in use (typical/atypical/combination/no medication)	N/A	22/23/9/1	N/A
Smoker/Non-smoker	7/50	28/39	0.0001
BPRS total score (range)	N/A	42.3 ± 7.7 (24–57)	N/A
BPRS negative score (range)	N/A	16.0 ± 4.9 (6–27)	N/A
BPRS positive score (range)	N/A	25.3 ± 6.3 (10–39)	N/A

Comparisons between groups were performed using χ^2 test for sex and smoking status, and t-test for age. Values are shown as means ± standard deviation (range). p-value in bold indicates statistically significant difference. BPRS, Brief Psychiatric Rating Scale; CPZ, chlorpromazine; N/A, not applicable. Typical antipsychotics included flufenazine, clorpromazine, amoxapine, haloperidol, perphenazine, thioridazine, and thiothixene. Atypical antipsychotics included clozapine, olanzapine, quetiapine, risperidone, ziprasidone, and aripiprazol.

We further evaluated whether glutamate and glutamine levels were associated with the severity of symptoms in patients with schizophrenia, measured by their scores on BPRS. Glutamate and glutamine levels were not significantly correlated to total BPRS score ($r = -0.07$, $p = 0.63$ for glutamate; $r = -0.04$, $p = 0.76$ for glutamine), to the BPRS score for negative symptoms ($r = -0.27$, $p = 0.06$ for glutamate; $r = 0.19$, $p = 0.18$ for glutamine), or to the BPRS score for positive symptoms ($r = 0.03$, $p = 0.84$ for glutamate; $r = -0.15$, $p = 0.31$ for glutamine).

DISCUSSION

The main findings of the present study are that, compared with healthy controls, patients with recent onset schizophrenia showed increased glutamine/glutamate ratio in the blood, while patients with chronic schizophrenia showed decreased glutamine/glutamate ratio. These findings are in agreement with one study that found decreased blood glutamate levels in first episode psychosis (27) and several studies including a meta-analysis that found increased levels of glutamate in the blood in patients with chronic schizophrenia compared with healthy controls (24, 25, 33). The evidence thus indicates that the glutamine/glutamate ratio is increased at the onset of schizophrenia but decreases with the progression of the disorder. Interestingly, the current use of antipsychotic medications (measured in chlorpromazine equivalents) was associated to the levels of glutamate and glutamine in chronic schizophrenia patients. However, the two cohorts showed opposite patterns of correlation: in Brazil more medication was associated to less glutamate and more glutamine, while in the USA patients taking more antipsychotic medications showed more glutamate and less glutamine. It is noteworthy that there were more patients in the Brazilian cohort taking typical antipsychotic (56%) than in the USA cohort (18%). Interestingly we found that patients using typical antipsychotic have lower glutamine/glutamate ratio than patients using atypical antipsychotics, indicating that different antipsychotic may have different effect on glutamate and glutamine levels. Accordingly, studies in patients and in animal models have been showing that some antipsychotic medication affects the release of glutamate in the brain, while others have no effect (37–39).

TABLE 5 | Blood levels of glutamate and glutamine in chronic schizophrenia (Brazil cohort) using smoking status as covariate.

Amino acids	Diagnostic	Mean	95% C.I.		Statistics
			Lower bound	Upper bound	
Glutamate, μmol/L	Healthy Control	264.5	191.8	337.2	$F = 16.3$, $p = 0.0001$
	Chronic schizophrenia	470.0	399.2	540.8	
Glutamine, μmol/L	Healthy Control	443.0	404.5	481.4	$F = 55.6$, $p = 0.0001$
	Chronic schizophrenia	242.3	204.8	279.8	
Glutamine/ glutamate ratio	Healthy Control	2.21	1.89	2.54	$F = 27.0$, $p = 0.0001$
	Chronic schizophrenia	1.02	0.71	1.34	

C.I., confidence interval.

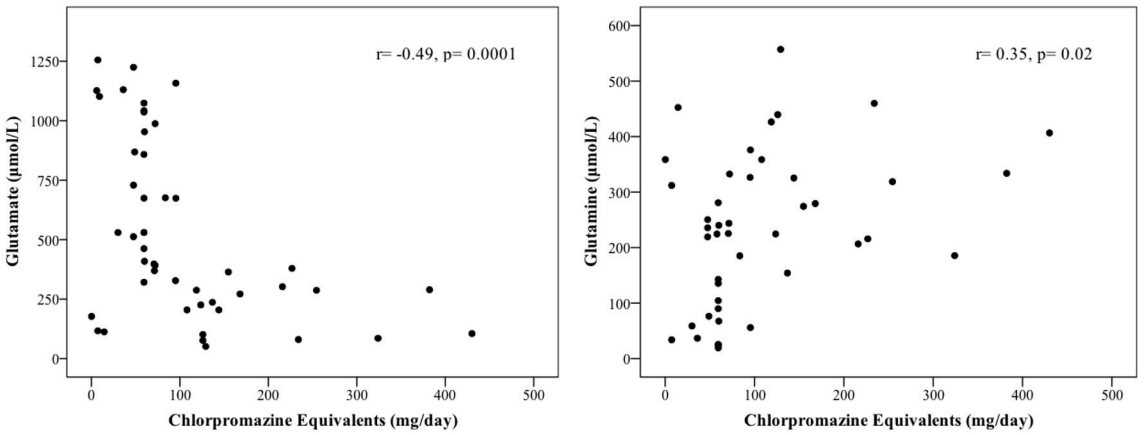

FIGURE 2 | Correlation between blood levels of glutamate and glutamine and the use of antipsychotic medication in chronic schizophrenia (Brazil cohort).

The increase in glutamate and the decrease in glutamine are in agreement with changes in the enzymes of the glutamate-glutamine cycle previously found in chronic schizophrenia. Studies in post-mortem brain tissue from patients with chronic schizophrenia found decreased levels of the glutamine synthetase protein (19, 20) and increased glutaminase expression and enzymatic activity (18, 21). The consequent decrease in glutamine synthesis and increase in glutamate formation by glutaminase may also explain the significant decrease in glutamine/glutamate ratio observed in patients with chronic schizophrenia compared to healthy controls.

It is important to consider whether the observed changes in glutamate and glutamine in schizophrenia patients could reflect processes occurring peripherally. Although most studies to date have shown positive correlations between blood and cerebrospinal fluid (CSF) levels of glutamate and glutamine (22, 40–43), other studies have reported lack of such correlations (37, 44). Moreover, one cannot exclude the possibility that differences in dietary habits in patients with schizophrenia may account for differences observed here. Patients with schizophrenia often have less healthy diets, consuming less fruit, and vegetables or more calories than the general population (45). Moreover, second-generation antipsychotics may induce altered eating behaviors, including increased susceptibility to hunger and changes in appetite perception (46).

Importantly, studies of glutamate and glutamine levels in the CSF of patients with schizophrenia suggest that our findings in the periphery reflect what is happening in the CNS (41, 43). While patients with chronic schizophrenia showed a significant increase in CSF glutamate levels compared to healthy controls, no difference was observed in patients with recent onset schizophrenia (41). More recently, Hashimoto et al. observed higher glutamine/glutamate ratio in the CSF in patients with recent onset schizophrenia when compared to normal controls (43). Although this argues against a peripheral source for the glutamate and glutamine changes we observed, we have no direct evidence to rule out this possibility.

A limitation of the present study is the absence of a longitudinal follow up of the patients to examine possible changes in glutamate and glutamine levels over time. Moreover, although the differences found in our cross-sectional study were highly significant, the cohorts studied were of modest size and they may not be representative of the population with schizophrenia as a whole.

In conclusion, we found increased glutamine/glutamate ratio in patients with recent onset schizophrenia while in chronic schizophrenia the glutamine/glutamate ratio was decreased, as compared to healthy controls. Given the putative role of dysfunctional glutamatergic neurotransmission in the pathophysiology of schizophrenia, further studies are warranted

to elucidate the underlying mechanisms and consequences of the alterations reported here.

AUTHOR CONTRIBUTIONS

All authors certify that they have participated sufficiently in the work to take public responsibility for the content. RP and SF participated in the conception and design of study. CM, RP, and SF wrote the manuscript. CM, CV-L, and RP performed the analysis and interpretation of the data. FA, MC, TS, FT, MF, NG, MB, and SV conducted analysis of patients.

ACKNOWLEDGMENTS

We thank A. C. Rangel and A. Fantinatti for administrative and technical support. This research was supported by grants from DECIT/SCTIE/MS, SESDC, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (RP and SF); Instituto Nacional de Ciência e Tecnologia de Biologia Estrutural e Bioimagem (INBEB) (RP); and National Institute for Translational Neuroscience (SF). RP was recipient of a Long-Term Fellowship from the Human Frontier Science Program. CM and CV-L were supported by pre-doctoral fellowships from FAPERJ and CNPq.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00713/full#supplementary-material

REFERENCES

1. Lee J, Park S. Working memory impairments in schizophrenia: a meta-analysis. *J Abnorm Psychol.* (2005) 114:599–611. doi: 10.1037/0021-843X.114.4.599
2. Bachman P, Reichenberg A, Rice P, Woolsey M, Chaves O, Martinez D, et al. Deconstructing processing speed deficits in schizophrenia: application of a parametric digit symbol coding test. *Schizophr Res.* (2010) 118:6–11. doi: 10.1016/j.schres.2010.02.1029
3. Olney JW, Farber NB. NMDA antagonists as neurotherapeutic drugs, psychotogens, neurotoxins, and research tools for studying schizophrenia. *Neuropsychopharmacology* (1995) 13:335–45. doi: 10.1016/0893-133X(95)00079-S
4. Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry* (2001) 158:1367–77. doi: 10.1176/appi.ajp.158.9.1367
5. Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R. Study of a new schizophrenomimetic drug; sernyl. *AMA Arch Neurol Psychiatry* (1959) 81:363–9. doi: 10.1001/archneurpsyc.1959.02340150095011
6. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humanspsychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* (1994) 51:199–214. doi: 10.1001/archpsyc.1994.03950030035004
7. Domino EF, Luby ED. Phencyclidine/schizophrenia: one view toward the past, the other to the future. *Schizophr Bull.* (2012) 38:914–9. doi: 10.1093/schbul/sbs011
8. Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* (2012) 37:4–15. doi: 10.1038/npp.2011.181
9. Maksymetz J, Moran SP, Conn PJ. Targeting metabotropic glutamate receptors for novel treatments of schizophrenia. *Mol Brain* (2017) 10:15. doi: 10.1186/s13041-017-0293-z
10. Gupta DS, McCullumsmith RE, Beneyto M, Haroutunian V, Davis KL, Meador-Woodruff JH. Metabotropic glutamate receptor protein expression in the prefrontal cortex and striatum in schizophrenia. *Synapse* (2005) 57:123–31. doi: 10.1002/syn.20164
11. Volk DW, Eggan SM, Lewis DA. Alterations in metabotropic glutamate receptor 1α and regulator of G protein signaling 4 in the prefrontal cortex in schizophrenia. *Am J Psychiatry* (2010) 167:1489–98. doi: 10.1176/appi.ajp.2010.10030318
12. Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized phase 2 clinical trial. *Nat Med*. (2007) 13:1102–7. doi: 10.1038/nm1632
13. Krystal JH, Abi-Saab W, Perry E, D'Souza DC, Liu N, Gueorguieva R, et al. Preliminary evidence of attenuation of the disruptive effects of the NMDA glutamate receptor antagonist, ketamine, on working memory by pretreatment with the group II metabotropic glutamate receptor agonist, LY354740, in healthy human subjects. *Psychopharmacology* (2005) 179:303–9. doi: 10.1007/s00213-004-1982-8
14. Ohtsuki T, Koga M, Ishiguro H, Horiuchi Y, Arai M, Niizato K, et al. A polymorphism of the metabotropic glutamate receptor mGluR7 (GRM7) gene is associated with schizophrenia. *Schizophr Res.* (2008) 101:9–16. doi: 10.1016/j.schres.2008.01.027
15. Mei YY, Wu DC, Zhou N. Astrocytic regulation of glutamate transmission in schizophrenia. *Front Psychiatry* (2018) 9:544. doi: 10.3389/fpsyt.2018.00544
16. Erecinska M, Silver IA. Metabolism and role of glutamate in mammalian brain. *Prog Neurobiol.* (1990) 35:245–96. doi: 10.1016/0301-0082(90)90013-7
17. Walton HS, Dodd PR. Glutamate-glutamine cycling in Alzheimer's disease. *Neurochem Int.* (2007) 50:1052–66. doi: 10.1016/j.neuint.2006.10.007
18. Gluck MR, Thomas RG, Davis KL, Haroutunian V. Implications for altered glutamate and GABA metabolism in the dorsolateral prefrontal cortex of aged schizophrenic patients. *Am J Psychiatry* (2002) 159:1165–73. doi: 10.1176/appi.ajp.159.7.1165
19. Burbaeva GSh, Boksha IS, Turishcheva MS, Vorobyeva EA, Savushkina OK, Tereshkina EB. Glutamine synthetase and glutamate dehydrogenase in the prefrontal cortex of patients with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* (2003) 27:675–80. doi: 10.1016/S0278-5846(03)00078-2
20. Steffek AE, McCullumsmith RE, Haroutunian V, Meador-Woodruff JH. Cortical expression of glial fibrillary acidic protein and glutamine synthetase is decreased in schizophrenia. *Schizophr Res.* (2008) 103:71–82. doi: 10.1016/j.schres.2008.04.032
21. Bruneau EG, McCullumsmith RE, Haroutunian V, Davis KL, Meador-Woodruff JH. Increased expression of glutaminase and glutamine synthetase mRNA in the thalamus in schizophrenia. *Schizophr Res.* (2005) 75:27–34. doi: 10.1016/j.schres.2004.12.012
22. Alfredsson G, Wiesel FA, Tylec A. Relationships between glutamate and monoamine metabolites in cerebrospinal fluid and serum in healthy volunteers. *Biol Psychiatry* (1988) 23:689–97. doi: 10.1016/0006-3223(88)90052-2
23. Hassan TH, Abdelrahman HM, Abdel Fattah NR, El-Masry NM, Hashim HM, El-Gerby KM, et al. Blood and brain glutamate levels in children with autistic disorder. *Res Autism Spectr Disord.* (2013) 7:541–8. doi: 10.1016/j.rasd.2012.12.005
24. Macciardi F, Lucca A, Catalano M, Marino C, Zanardi R, Smeraldi E. Amino acid patterns in schizophrenia: some new findings. *Psychiatry Res.* (1990) 32:63–70. doi: 10.1016/0165-1781(90)90136-S
25. Ivanova SA, Boyko AS, Fedorenko OY, Krotenko NM, Semke AV, Bokhan NA. Glutamate concentration in the serum of patients with schizophrenia. *Proc Chem.* (2014) 10:80–5. doi: 10.1016/j.proche.2014.10.015

26. Alfredsson G, Wiesel FA. Monoamine metabolites and amino acids in serum from schizophrenic patients before and during sulpiride treatment. *Psychopharmacology* (1989) 99:322–7. doi: 10.1007/BF00445551
27. Palomino A, González-Pinto A, Aldama A, González-Gómez C, Mosquera F, González-García G, et al. Decreased levels of plasma glutamate in patients with first-episode schizophrenia and bipolar disorder. *Schizophr Res.* (2007) 95:174–8. doi: 10.1016/j.schres.2007.06.012
28. He Y, Yu Z, Giegling I, Xie L, Hartmann AM, Prehn C, et al. Schizophrenia shows a unique metabolomics signature in plasma. *Transl Psychiatry* (2012) 2:e149. doi: 10.1038/tp.2012.76
29. Fisher M, Holland C, Merzenich MM, Vinogradov S. Using neuroplasticity-based auditory training to improve verbal memory in schizophrenia. *Am J Psychiatry* (2009) 166:805–11. doi: 10.1176/appi.ajp.2009.08050757
30. Vinogradov S, Fisher M, Holland C, Shelly W, Wolkowitz O, Mellon SH. Is serum brain-derived neurotrophic factor a biomarker for cognitive enhancement in schizophrenia? *Biol Psychiatry* (2009) 66:549–53. doi: 10.1016/j.biopsych.2009.02.017
31. Vinogradov S, Fisher M, Warm H, Holland C, Kirshner MA, Pollock BG. The cognitive cost of anticholinergic burden: decreased response to cognitive training in schizophrenia. *Am J Psychiatry* (2009) 166:1055–62. doi: 10.1176/appi.ajp.2009.09010017
32. Leucht S, Rothe P, Davis JM, Engel RR. Equipercentile linking of the BPRS and the PANSS. *Eur Neuropsychopharmacol.* (2013) 23:956–9. doi: 10.1016/j.euroneuro.2012.11.004
33. Song J, Viggiano A, Monda M, De Luca V. Peripheral glutamate levels in schizophrenia: evidence from a meta-analysis. *Neuropsychobiology* (2014) 70:133–41. doi: 10.1159/000364828
34. Hashimoto A, Nishikawa T, Oka T, Takahashi K, Hayashi T. Determination of free amino acid enantiomers in rat brain and serum by high-performance liquid chromatography after derivatization with N-tert-butyloxycarbonyl-L-cysteine and o-phthaldialdehyde. *J Chromatogr.* (1992) 582:41–8. doi: 10.1016/0378-4347(92)80300-F
35. Calcia MA, Madeira C, Alheira FV, Silva TC, Tannos FM, Vargas-Lopes C, et al. Plasma levels of D-serine in Brazilian individuals with schizophrenia. *Schizophr Res.* (2012) 142:83–7. doi: 10.1016/j.schres.2012.09.014
36. Sang KK, Jong H. Statistical data preparation: management of missing values and outliers. *Korean J Anesthesiol.* (2017) 70:407–11. doi: 10.4097/kjae.2017.70.4.407
37. Goff DC, Hennen J, Lyoo IK, Tsai G, Wald LL, Evins AE, et al. Modulation of brain and serum glutamatergic concentrations following a switch from conventional neuroleptics to olanzapine. *Biol Psychiatry* (2002) 5:493–7. doi: 10.1016/S0006-3223(01)01321-X
38. Sacchi S, Novellis V, Paolone G, Nuzzo T, Iannotta M, Belardo C, et al. Olanzapine, but not clozapine, increases glutamate releasein the prefrontal cortex of freely moving mice by inhibiting D-aspartate oxidase activity. *Sci Rep.* (2017) 7:46288. doi: 10.1038/srep46288
39. Egerton A, Bhachu A, Merritt K, McQueen G, Szulc A, McGuire P. Effects of antipsychotic administration on brain glutamate in schizophrenia: a systematic review of longitudinal 1H-MRS studies. *Front Psychiatry* (2017) 8:66. doi: 10.3389/fpsyt.2017.00066
40. McGale EH, Pye IF, Stonier C, Hutchinson EC, Aber GM. Studies of the inter-relationship between cerebrospinal fluid and plasma amino acid concentrations in normal individuals. *J Neurochem.* (1977) 29:291–7. doi: 10.1111/j.1471-4159.1977.tb09621.x
41. Gattaz WF, Gattaz D, Beckmann H. Glutamate in schizophrenics and healthy controls. *Arch Psychiatr Nervenkr.* (1982) 231:221–5. doi: 10.1007/BF00343292
42. Alfredsson G, Wiesel FA, Lindberg M. Glutamate and glutamine in cerebrospinal fluid and serum from healthy volunteers–analytical aspects. *J Chromatogr.* (1988) 424:378–84. doi: 10.1016/S0378-4347(00)81116-0
43. Hashimoto K, Engberg G, Shimizu E, Nordin C, Lindström LH, Iyo M. Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients. *BMC Psychiatry* (2005) 5:6. doi: 10.1186/1471-244X-5-6
44. Shulman Y, Grant S, Seres P, Hanstock C, Baker G, Tibbo P. The relation between peripheral and central glutamate and glutamine in healthy male volunteers. *J Psychiatry Neurosci.* (2006) 31:406–10.
45. Bushe C, Haddad P, Peveler R, Pendlebury J. The role of lifestyle interventions and weight management in schizophrenia. *J Psychopharmacol.* (2005) 19:28–35. doi: 10.1177/0269881105058682
46. Blouin M, Tremblay A, Jalbert ME, Venables H, Bouchard RH, Roy MA, et al. Adiposity and eating behaviors in patients under second generation antipsychotics. *Obesity* (2008) 16:1780–7. doi: 10.1038/oby.2008.277

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 10 January 2019
doi: 10.3389/fpsyt.2018.00733

Treatment Responses of Cognitive Function and Plasma Asymmetric Dimethylarginine to Atypical Antipsychotic in Patients With Schizophrenia

Zhi-min Yu[1†], *Ying Zhao*[2†], *Jin-qiong Zhan*[3†], *Tao Luo*[1], *Jian-wen Xiong*[1], *Bin Yu*[1], *Bo Wei*[1*] and *Yuan-jian Yang*[1,3*]

[1] Department of Psychiatry, Jiangxi Mental Hospital/Affiliated Mental Hospital of Nanchang University, Nanchang, China, [2] Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [3] Biological Psychiatry Laboratory, Jiangxi Mental Hospital/Affiliated Mental Hospital of Nanchang University, Nanchang, China

OPEN ACCESS

Edited by:
Hsien-Yuan Lane,
China Medical University, Taiwan

Reviewed by:
Rogerio Panizzutti,
Universidade Federal do Rio de Janeiro, Brazil
Chih-Min Liu,
National Taiwan University Hospital, Taiwan

***Correspondence:**
Bo Wei
jxmh_wb@163.com
Yuan-jian Yang
yuanjimyang@yeah.net

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: 02 November 2018
Accepted: 13 December 2018
Published: 10 January 2019

Citation:
Yu Z, Zhao Y, Zhan J, Luo T, Xiong J, Yu B, Wei B and Yang Y (2019) Treatment Responses of Cognitive Function and Plasma Asymmetric Dimethylarginine to Atypical Antipsychotic in Patients With Schizophrenia. Front. Psychiatry 9:733. doi: 10.3389/fpsyt.2018.00733

Cognitive deficits represent a core feature of schizophrenia. Previous studies have demonstrated that plasma asymmetric dimethylarginine (ADMA) was increased in patients with schizophrenia and correlated with cognitive impairments. Atypical antipsychotics can produce cognitive benefits in schizophrenia patients. In this study, we conducted a prospective observation trial to explore whether plasma ADMA may serve as an indicator for evaluating cognitive improvements induced by atypical antipsychotics in patients with schizophrenia. A total of 41 schizophrenia patients with acute exacerbation were enrolled and 29 patients completed this study. These recruited patients were drug-naive or had no exposure to antipsychotics for at least 3 months. Thirty healthy individuals were recruited as a control group. Positive and Negative Syndrome Scale (PANSS) and a neuropsychological battery were used to evaluate schizophrenic symptoms and cognitive function, respectively. Plasma ADMA was measured by high-performance liquid chromatography (HPLC). We found that schizophrenia patients with acute exacerbation had significantly poorer cognitive performances and higher plasma ADMA levels than control individuals ($p < 0.05$). After 2 months of atypical antipsychotic treatment, patients showed significant improvements in processing speed, working memory, attention, and executive function (all $p < 0.01$). Plasma ADMA levels in patients after treatment were significantly decreased compared to baseline (2.42 ± 0.84 vs. 1.55 ± 0.34 μmol/L; $t = 6.491$, $p < 0.001$). Correlation analysis reveals that there is a significant correlation of the decrease in ADMA with improvements in working memory ($r = -0.413$, $p = 0.026$) and attention ($r = -0.417$, $p = 0.025$). Collectively, our results suggest that atypical antipsychotics improve cognitive function in schizophrenia patients with acute exacerbation, in parallel with decreased plasma ADMA levels. Plasma ADMA levels may be an indicator of cognitive recovery in schizophrenia.

Keywords: schizophrenia, cognitive function, asymmetric dimethylarginine (ADMA), atypical antipsychotic, plasma

INTRODUCTION

Schizophrenia is a chronic mental disorder with a prevalence of ∼1% in the population worldwide. Along with positive and negative symptoms, deficits of cognitive function are also deemed as a core feature of schizophrenia (1, 2). A large number of cognitive areas including processing speed, attention, visual memory, verbal learning, working memory, and execute capacity have been reported to be impaired in patients with schizophrenia (3, 4). They are present before the onset of psychosis and may lead to functional disability, including defects in work and social contact, independent living, as well as skill acquirement (5, 6). Thus, the treatment of cognitive deficits has great benefits for the clinical outcome of patients with schizophrenia.

Antipsychotic drug treatment has been recommended to be a key component of schizophrenia treatment algorithms (7). First-generation antipsychotics (FGAs) were discovered in the 1950s and have therapeutic efficacy for positive symptoms, including delusions and hallucinations (8). However, long-term treatment with FGAs can cause a side-effect of cognitive deterioration (9). Since second-generation drugs (SGAs), known as atypical antipsychotics, have been developed and introduced clinically in the 1970s, they have been more frequently used for the management of schizophrenia in recent decades. In addition to positive symptoms, atypical antipsychotics also have significant impacts on negative symptoms and cognitive deficits associated with schizophrenia (9, 10). Numerous double-blind, random-controlled clinical studies have shown that, in compared to FGAs, treatment of atypical antipsychotics could greatly promote cognitive capability in patients with schizophrenia (9–11). Overall cognitive ability and specific domains of cognition such as processing speed, memory, and attention were improved after the usage of atypical antipsychotics (10, 12).

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase (NOS). It is physiologically generated during the methylation of protein arginine residues and released during proteolysis (13). Previous studies have demonstrated that plasma ADMA concentrations were increased in patients with schizophrenia relative to healthy individuals, but no association was found between plasma ADMA and the scores of psychiatric rating scales (14–16). We also found that plasma ADMA levels were elevated in schizophrenia patients and increased ADMA correlated with cognitive impairment (17). Zincir et al. reported that treatment with antipsychotics in patients for 2 months could reduce plasma ADMA levels, while no correlation was observed between the improvement of psychiatric symptoms and the change of plasma ADMA (16). However, whether there is a relationship between the reduction of plasma ADMA and cognitive improvement in patients treated with atypical antipsychotics remains unknown.

In this study, we hypothesized that peripheral ADMA levels may be a potential indicator of cognitive recovery in schizophrenia. To test this hypothesis, we investigated whether (1) eight-week atypical antipsychotic monotherapy improves cognition in schizophrenia patients with acute exacerbation; (2) peripheral ADMA is decreased at the endpoint (8th-week) compared to baseline; and (3) improvement of cognition is correlated with change of ADMA levels.

MATERIALS AND METHODS

Subjects

This is an open-label prospective observation study. All patients in this study were recruited from Jiangxi Mental Hospital. The patients of schizophrenia were diagnosed by two trained research psychiatrists using modified sections of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The age of recruited patients was between 18 and 50 years old and the Positive and Negative Syndrome Scale (PANSS) score need to be between 60 and 120 at the baseline interview. All the patients who were recruited in this study had acute exacerbation for their psychotic symptoms and were admitted into acute ward for assessment and treatment. All these patients were antipsychotic naive or had not taken any antipsychotic drug over the past 3 months. In order to reassure the drug compliance, only patients who stayed well for at least 8-weeks were included in the present study. Healthy individuals were recruited from the local community as controls, matched with the patients by age, gender, education, and body mass index (BMI). All participants were Han Chinese ethnicity.

Patients with neurological disorders, mental retardation, and drug/alcohol abuse or addiction were excluded from this study. Some diseases including metabolic syndrome, diabetes, hyperlipidemia, coronary artery disease, inflammatory disease, liver damage, and renal failure may lead to changes in plasma ADMA (18–23). Thus, besides any additional axis I or axis II DSM-IV diagnosis, the exclusion criteria also included acute or chronic infections, current pregnancy, autoimmune, endocrine, and neoplastic diseases, allergic asthma, and other acute physical disorders, including heart or brain infarction within the past 3 months. Both patient and control groups were assessed with a medical history, physical examination, electrocardiogram, and laboratory tests. They were screened for acute infectious diseases by measuring body temperature, erythrocyte sedimentation rate, serum creatinine, C-reactive protein (CRP), and urinary culture. Subjects with abnormal parameters were excluded, for example urea (Normal range: 2.14∼7.85 mmol/L), creatinine (Normal range: 60∼130 μmol/L), SGOT (Normal range: 0∼40 U/L), SGPT (Normal range: 0∼40 U/L), CRP (Normal range: 0∼10 mg/L), cholesterol (Normal range: 3.38∼6.47 mmol/L), triglycerides (Normal range: 0.4∼1.81 mmol/L), systolic and diastolic blood pressure (Normal range: 140/90-90/60 mmHg), and BMI (Normal range: 18.5∼24).

All procedures for this study were reviewed and approved by the Institutional Review Board at Jiangxi Mental Hospital. The research was carried out in accordance with the Declaration of Helsinki and other relevant national and international regulations. A written informed consent was obtained from each subject, or his or her legal guardians.

Evaluations of Clinical Symptom and Cognitive Function

PANSS was used to assess the severity of the psychotic symptoms of patients. The evaluation was conducted by two psychiatrists who had both attended a training session of PANSS use before the study began. The inter-observer correlation coefficient for the PANSS total score was >0.80.

The cognitive function of subjects was measured by a comprehensive battery of neurocognitive tests (17, 24, 25). These tests are commonly used in China, and their clinical reliability and validity have been evaluated in Chinese populations. The battery consists of seven tests and can be grouped into six cognitive domains: processing speed (Trail making test part A: TMT-A; Brief assessment of cognition in schizophrenia-symbol coding: BACS-symbol coding), working memory (Wechsler memory scale-3rd edition-spatial span: WMS-III spatial span), visual memory (Brief visual-spatial memory test-revised: BVMT-R), verbal learning (Hopkins verbal learning test-revised: HVLT-R), attention (Continuous performance test-identical pairs: CPT-IP), and executive function (Stroop color-word test).

TMT-A: In this test, you are given a pencil and a piece of paper with numbered circles, and you are asked to draw a line and connect the circles in the sequence of their numbers. You should draw the lines as quickly and as accurately as you can, since the time taken for the drawing is used for grading.

BACS-symbol coding: In this test, you are given 133 pairs of digits and symbols, and asked to copy the specific symbol as soon as you are shown its paired number. You should do this quickly as the symbols you finish correctly within 120 s are used for the grading.

WMS-III space span: In this test you are faced with a board with ten cubes on it spaced irregularly. An administrator will firstly show you combinations of the cubes in different ways and orders, forward and backward, and then ask you to recall the combinations. On each level of combination, you are given two trials. In the end you are graded in line with your recalled trials.

BVMT-R: In this test you will watch six geometric figures, which will appear three times, each time for 10 s. After that you are asked to draw the figures on a piece of paper in the layout as you watched. The more figures you draw correctly, the better you are rated.

HVLT-R: In this test, you will be presented with twelve Chinese words, which are listed in three categories. The list will be shown three times, followed by a delay time of 25–30 min. After that, you are asked to recall and speak out the words. Your performance is graded in accordance with the words you correctly recalled.

CPT-IP: In this test, you will see digital numbers of 2, 3, and 4 digits flashing on a computer screen. You are asked to immediately click the mouse after you see the same number flash on the screen in a row. In this test, you may hit 90 times in total, and possibly trigger false alarms 90 times in total. You will randomly respond about 270 times in total.

Stroop color-word test: In this test you're presented with three pages: firstly a word page, on which words of colors are black in color, secondly a color page, on which several rows of Xs are in different colors and thirdly a word-color page, on which the same words of colors on the first page are colored using the colors on the second page, though they are not colored in the color of themselves. In each trial you are presentedwith 100 words and you are asked to read them as fast as you can in a cycle time of 45 s. Your correct readings of the words are numbered for your grading.

Plasma ADMA Level Analysis by High-Performance Liquid Chromatography (HPLC)

Peripheral blood was collected from an antecubital vein between 07:00 and 09:00 a.m. following an overnight fasting at baseline and at the endpoint of the study. The blood samples were centrifuged at 3,000 rpm for 5 min at 4°C. The plasma was separated and stored at −80°C until analysis.

The concentration of plasma ADMA was measured by HPLC as described in our previous study (17). Briefly, we added 5 mg of 5-sulphosalici-licacid (SSA) to 1 mL of plasma and allowed them to mix for 10 min at −20°C. The sample was then centrifuged at 7,000 rpm for 5 min at temperature of 4°C. After that, 10 microliters of the supernatant was mixed with 100 μL of derivatization reagent (prepared by dissolving 10 mg of o-phthaldialdehyde in 0.5 mL of methanol, and adding 2 mL of 0.4 M borate buffer (pH 10.0) and 30 μL of 2-mercaptoethanol). The mixture was subsequently injected into the chromatographic system to determine the level of ADMA. The variability of this method was <7%, and the detection limit of the assay was 0.1 μm.

Statistical Analysis

Demographic and clinical variables were compared between control and patient groups using Student's *t*-test or analysis of variance (ANOVA) for quantitative variables and chi-squared test for qualitative variables. An analysis of covariance (ANCOVA) was performed to compare the scores of cognitive tests between two groups, controlling for gender, age, years of education, and BMI by using these variables as covariates. To compare the levels of ADMA between the patient and control groups, ANCOVA was performed using gender, age, years of education, and BMI as covariates to minimize the potential effect of these factors on the expression of ADMA levels. A paired *t*-test was performed to analyze plasma concentrations of ADMA between two time points (at baseline and 8th-week after atypical antipsychotic treatment) in the same patients. A Non-parametric test (Kruskal-Wallis test) was used to test whether there was a difference in cognitive function and ADMA level among different atypical antipsychotics at the endpoint. Spearman's correlation analysis was used to examine the correlation between change of cognitive test scores and ADMA level. All statistical tests were two-tailed, and the significance level was set at $p < 0.05$.

RESULTS

Demographic and Clinical Characteristics and Cognitive Performance

A total of 41 schizophrenia patients with acute exacerbation consented to the study and received baseline assessments. During the study session, these patients only received one type of atypical antipsychotics. The type of atypical antipsychotic was chosen according to the demographic and clinical symptom characteristics of patients. Patients using clozapine met the criteria of refractory schizophrenia. Benzene hydrochloride was only used when patients developed a marked extrapyramidal response. Of these, 12 patients dropped out for the following

reasons: four patients were discharged from hospital before the endpoint of study; five patients did not respond to atypical antipsychotic monotherapy and required a combined treatment with other antipsychotic drugs or modified electric convulsive treatment (MECT); three patients suffered severe impairment of liver function and need to receive liver-protecting therapy. All data reported here are based on the remaining 29 patients who completed this study.

Baseline demographic and clinical characteristics for the patient and control groups are shown in **Table 1**. There was no significant difference in age, gender, years of education, or BMI between the two groups (all $p > 0.05$). Cognitive tests revealed that schizophrenia patients with acute exacerbation demonstrated significantly poorer cognitive performance than control individuals except for the BVMT-R controlling for age, gender, years of education, and BMI (all $p < 0.05$). We then measured the levels of plasma ADMA in both patient and control groups. The data of 29 patients and 30 healthy controls were included in the analysis of baseline ADMA levels. As previously reported (16, 17), patients with schizophrenia showed higher levels of plasma ADMA relative to the controls (2.42 ± 0.84 vs. 1.41 ± 0.14 μmol/L; $F = 42.947$, $p < 0.001$). ANCOVA analysis revealed that the difference between two groups still existed ($F = 10.098$, $p < 0.001$) when age, gender, years of education, and BMI were used as covariates.

TABLE 1 | Baseline demographic and clinical characteristics for patient and control groups.

	Patients	Controls	$F/\chi 2$	p
Age (years)	31.17 ± 8.19	32.03 ± 8.25	0.162	0.689
Gender (M/F)	14/15	16/14	0.151	0.797
Education (years)	9.79 ± 4.90	11.73 ± 5.22	2.163	0.147
BMI (kg/m^2)	21.43 ± 1.78	20.93 ± 1.87	1.110	0.297
Duration of illness (years)	6.82 ± 5.56	NA		
PANSS				
Total scores	80.14 ± 8.21	NA		
Positive subscore	23.10 ± 4.72	NA		
Negative subscore	14.14 ± 5.13	NA		
General psychopathology	43.90 ± 4.15	NA		
COGNITIVE FUNCTION				
TMT-A	69.48 ± 23.37	40.37 ± 10.33	7.511	<0.001
BACS-SC	31.52 ± 11.03	65.63 ± 5.93	43.472	<0.001
WMS-III-SS	13.92 ± 2.86	17.62 ± 3.31	5.311	<0.001
HVLT-R	20.17 ± 6.72	26.27 ± 5.37	3.247	0.012
BVMT-R	21.41 ± 5.20	26.70 ± 9.29	2.314	0.066
CPT-IP	1.34 ± 0.88	3.46 ± 1.03	14.175	<0.001
Stroop word score	52.79 ± 13.98	85.87 ± 8.15	26.982	<0.001
Stroop color score	31.24 ± 14.29	50.57 ± 8.93	7.633	<0.001
Stroop color-word score	19.72 ± 11.14	37.10 ± 8.05	10.449	<0.001
Plasma ADMA (μmol/L)	2.42 ± 0.84	1.41 ± 0.14	10.098	<0.001

BMI, body mass index; PANSS, Positive and Negative Syndrome Scale; TMT-A, trail making task part A; BACS-SC, brief assessment of cognition in schizophrenia-symbol coding; CPT-IP, continuous performance test-identical pairs; WMS-III-SS, Wechsler memory scale-3rd edition-spatial span; HVLT-R, Hopkins verbal learning test-revised; BVMT-R, brief visual-spatial memory test-revised. NA, not applicable.

Clinical Efficacy and Neurocognitive Effect of Atypical Antipsychotic in Patients

The primary endpoint was the change from baseline to 8-weeks in PANSS and cognitive test scores. The number of patients treated with risperidone, olanzapine, clozapine, and aripiprazole was 10, 5, 7, and 7, respectively. The final median dose of risperidone, olanzapine, clozapine, and aripiprazole was 3.12 ± 1.05, 16.27 ± 5.33, 292.75 ± 115.63, and 14.66 ± 3.81 mg/day, respectively. **Table 2** shows the clinical efficacy and neurocognitive effect of atypical antipsychotics in schizophrenia patients with acute exacerbation. A significant improvement in PANSS total score and each subscore was observed when the patients completed this study (Paired t-tests; all $p < 0.01$). According to the proposed criteria for symptomatic remission in patients with schizophrenia (26), the rate of symptomatic remission at the endpoint was 82.75% (24/29). Additionally, patients given an 8-week period of atypical antipsychotic treatment showed significant improvements in the test scores of TAM-A, BACS-SC, WMS-III, CPT-IP, and Stroop color-word test (all $p < 0.01$). Furthermore, Kruskal-Wallis test showed that there was no difference in PANSS total score, each subscore, or the cognitive tests mentioned above, among risperidone, olanzapine, clozapine, and aripiprazole-treated groups at the endpoint ($p > 0.05$).

Plasma ADMA Level After Atypical Antipsychotic Treatment

Together with clinical improvement, plasma ADMA levels after 8-week atypical antipsychotic monotherapy were also significantly decreased compared with baseline (2.42 ± 0.84 vs. 1.55 ± 0.34 μmol/L; $t = 6.491$, $p < 0.001$) (**Figure 1**). There was no significant difference in plasma ADMA levels between the patients after atypical antipsychotic treatment and the controls (1.55 ± 0.34 vs. 1.41 ± 0.14 μmol/L; $p > 0.05$). Furthermore, all these atypical antipsychotics could lead to a significant decrease in the levels of plasma ADMA ($p < 0.05$), and there was no significant difference between groups in plasma ADMA levels at the study endpoint (Kruskal-Wallis test, $\chi^2 = 4.255$, $p = 0.235$) (**Table 3**).

Relationship Between Cognitive Improvement and Change of Plasma ADMA Levels

Spearman's correlation analysis was conducted to explore whether the change in plasma ADMA levels was correlated with clinical improvements. Similar to a previous study (16), we did not find any relationship between the reduction of plasma ADMA and the change of PANSS total score ($r = 0.028$, $p = 0.886$), positive subscore ($r = -0.021$, $p = 0.913$), negative subscore ($r = -0.044$, $p = 0.821$), or general psychopathology ($r = 0.055$, $p = 0.776$). Then we further analyzed the relationship between the change of plasma ADMA levels and cognitive improvement. As shown in **Figure 2**, there is a significant correlation between the decrease of ADMA and the change in WMS-III ($r = -0.413$,

TABLE 2 | Comparison of PANSS and cognitive test scores between baseline and endpoint in patients.

	Baseline	Endpoint	*t*	*p*
PANSS				
Total scores	80.14 ± 8.21	36.14 ± 9.27	29.653	<0.001
Positive subscore	23.10 ± 4.72	7.86 ± 2.29	19.921	<0.001
Negative subscore	14.14 ± 5.13	7.72 ± 1.23	9.149	<0.001
General psychopathology	43.90 ± 4.15	20.55 ± 6.09	18.517	<0.001
COGNITIVE FUNCTION				
TMT-A	69.48 ± 23.37	51.83 ± 17.06	5.324	<0.001
BACS-SC	31.52 ± 11.03	43.21 ± 12.41	−4.393	<0.001
WMS-III-SS	13.92 ± 2.86	17.08 ± 3.52	−5.556	<0.001
HVLT-R	20.17 ± 6.72	23.14 ± 11.13	−1.995	0.056
BVMT-R	21.41 ± 5.20	23.66 ± 8.01	−1.641	0.112
CPT-IP	1.34 ± 0.88	1.87 ± 0.63	−2.809	0.009
Stroop word score	52.79 ± 13.98	74.31 ± 12.65	−7.093	<0.001
Stroop color score	31.24 ± 14.29	44.28 ± 12.48	−4.379	<0.001
Stroop color-word score	19.72 ± 11.14	29.52 ± 12.69	−4.937	<0.001

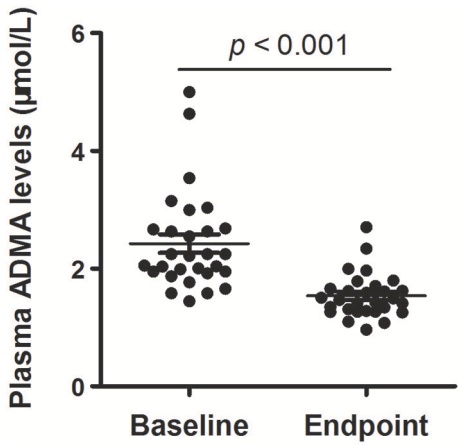

FIGURE 1 | Plasma ADMA levels of schizophrenia patients with acute exacerbation at baseline and endpoint (8th-week). Scatter plot for the comparison of plasma ADMA levels at baseline and endpoint. The sample means are indicated by the black bars.

$p = 0.026$) or CPT subscore ($r = -0.417$, $p = 0.025$), indicating that the decrease in plasma ADMA was correlated with the improvements of working memory and attention in patients treated with atypical antipsychotic.

DISCUSSION

This is a prospective, open-label, 8-week observational study, aiming to explore the efficacy of atypical antipsychotics on cognitive deficits in patients with schizophrenia. We found that schizophrenia patients with acute exacerbation displayed multi-faceted cognitive impairments in comparison with healthy individuals. Biologically, plasma levels of ADMA were much higher in patients than in controls. 8-week treatment with atypical antipsychotic significantly improved psychiatric symptoms and cognitive function, as well as leading to decreased plasma ADMA levels in patients. The decrease in ADMA levels is significantly associated with cognitive improvement of patients.

TABLE 3 | Plasma ADMA levels before and after treatment.

	Baseline ADMA (μmol/L)	Endpoint ADMA (μmol/L)	*t*	*p*
Risperidone (n =10)	2.08 ± 0.48	1.46 ± 0.29	3.946	0.003
Olanzapine (n = 5)	2.35 ± 0.68	1.42 ± 0.13	2.857	0.046
Clozapine (n = 7)	2.80 ± 1.06	1.73 ± 0.57	3.965	0.007
Aripiprazole (n = 7)	2.59 ± 1.03	1.61 ± 0.01	2.571	0.042
$\chi 2$	3.102	4.255		
p	0.376	0.235		

Cognitive deficits are a core feature of schizophrenia. Consistent with previous reports (17, 24, 25), our present study reveals that schizophrenia patients with acute exacerbation exhibited a wide range of cognitive impairments across multiple domains including processing speed, working memory, attention, verbal learning, and executive capability. Treatment with risperidone, olanzapine or aripiprazole could improve specific cognitive domains with similar global clinical efficacy in schizophrenia patients (10, 12). Results from animal experiments also showed that these atypical antipsychotics had positive benefits for cognitive deficits in a rat model of schizophrenia (27–29). In the present study, the recruited patients received medication of risperidone, olanzapine, clozapine or aripiprazole, and they showed significant improvements in cognitive domains of processing speed, working memory, attention, and executive function after 2 months of treatment. These results demonstrate the efficacy of atypical antipsychotics in cognitive impairments of schizophrenia patients with acute exacerbation.

Nitric oxide (NO) is a gas messenger that exerts multiple biological effects in the central nervous system. Deficits of NO signaling are shown to be linked with the pathogenesis of schizophrenia (30, 31). For example, Reif et al. reported that regulatory polymorphisms of NOS contributed to the genetic risk for schizophrenia (32). A decrease in nitrergic neurons was found in striatum of schizophrenia patients (33). The levels of NO and its metabolites were decreased in serum or plasma of patients with schizophrenia (14, 34). As an endogenous competitive inhibitor of NOS, ADMA was increased in the plasma of schizophrenia patients (16, 17). Treating schizophrenia patients with antipsychotic for 2 months led to a significant reduction in plasma ADMA levels (16). Consistent with this report, our present study showed that the levels of plasma ADMA in patients after 8-week atypical antipsychotic treatment were significantly decreased compared to that in controls. No difference was found in ADMA levels among patients-treated with risperidone, olanzapine, clozapine or aripiprazole at the study endpoint, demonstrating similar efficacy of these antipsychotics in the regulation of plasma ADMA levels in patients.

NO plays an essential role in synaptic plasticity and cognition (35, 36). Inhibition of endogenous NO generation impairs

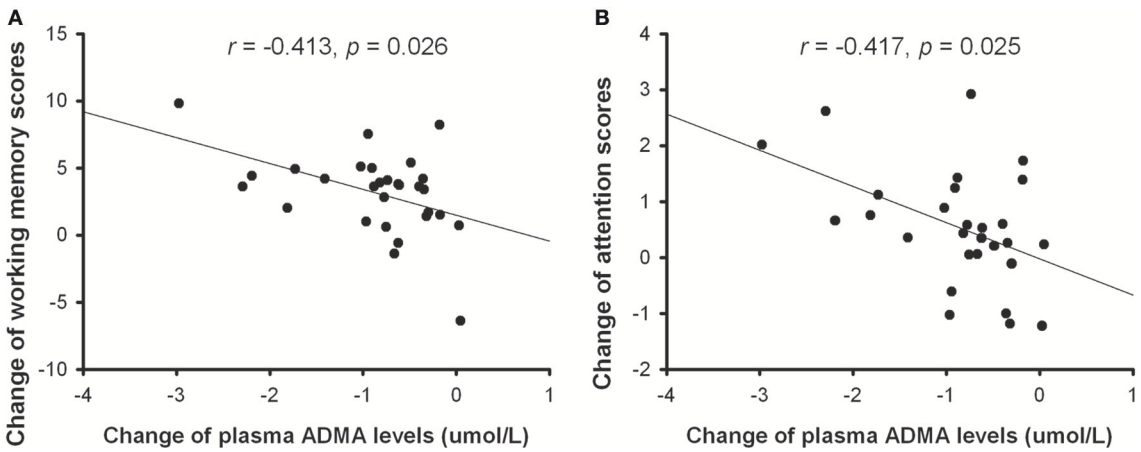

FIGURE 2 | The correlation between the change of plasma ADMA levels and improvement of working memory **(A)** or attention **(B)** in patients with atypical antipsychotic treatment.

synaptic plasticity and learning and memory (37–39). As an endogenous inhibitor of NOS, ADMA decreases the levels of NO by inhibition of NOS activity (13). Our previous study found that increased plasma ADMA was associated with cognitive deficits in patients with schizophrenia (17). The results of our present study showed that atypical antipsychotic treatment could significantly decrease plasma ADMA levels and parallelly improve cognitive function in schizophrenia patients with acute exacerbation. A series of studies have demonstrated the efficacy of NO on cognitive impairments in animal models of schizophrenia (31). Specifically, central supplement of S-nitroso-N-acetylpenicillamine (SNAP), an NO donor, attenuated the impaired spatial memory in MK-801-treated mice (40). Intraperitoneal injection of GT 1061, a novel nitrate that enhance NO release, reversed MK-801-induced reversal learning deficits in rats (41). Treatment with sodium nitroprusside attenuated the attention impairment produced by amphetamine in the prepulse inhibition test in mice (42). In view of the role of NO in schizophrenia-related cognitive deficits and inhibition of endogenous ADMA on NO signaling, we postulated that atypical antipsychotic may exert a beneficial effect on cognitive function by upregulating NO signaling in patients with schizophrenia. However, further investigations are needed to address this postulation.

An interesting finding of this study is that improvement of cognitive function was associated with decreased ADMA levels, in patients treated with atypical antipsychotic. This finding indicates that the plasma ADMA level may be a potential indicator for evaluating the outcome for cognitive deficits after atypical antipsychotic treatment. Although underproduction of NO has been shown to contribute to the pathology of schizophrenia and decreased levels of plasma NO have been found in schizophrenia patients (14, 31), NO is not suitable to be a biomarker because it has a very short period of existence in plasma. ADMA levels are relatively stable and can be accurately measured in plasma (13). Thus, it may be more suitable to serve as a peripheral biomarker than NO. However, due to the small sample size of this study, further large-scale clinical studies are needed to confirm the value of plasma ADMA as an indicator for cognitive evaluation in schizophrenia.

Despite the suggestive results, some limitations of the present study should be noted. First, this is an open-label prospective observational study and selection bias may have been introduced. Generalization of these findings beyond the population should be cautious. Second, we measured the level of ADMA in plasma. Whether plasma ADMA can reflect a similar change in the brain is still uncertain. Third, each type of atypical antipsychotics may produce cognitive benefits in specific cognitive domains (12). However, the sample size of this study is small and limits further analysis of the correlation between improvement of specific cognitive domain and ADMA decrease stratified by type of atypical antipsychotics. Thus, large-scale clinical studies are needed to replicate and verify these findings. Fourth, Spearman's correlation analysis reveals a relationship between cognitive improvement and ADMA decrease in patients with schizophrenia. However, a causal relationship between the two variables cannot be drawn. Further research using animal experiments is needed to address this issue. Fifth, the symptomatic remission rate found in our present study (82.75%) exceeded reported rates (16 to 78%) (43). Perhaps the type of antipsychotics and the criteria for data collection could partially explain the difference. All the patients recruited in this study were treated with atypical antipsychotics, which might improve the treatment compliance of patients and thus resulted in a higher remission rate. In addition, as patients who did not respond to atypical antipsychotic monotherapy and required combined treatment with other antipsychotic or MECT were considered as dropout and were not included in the data analysis, this might also have contributed to the high statistical value for theremission rate. The sample characteristics of this study limit the generalization of the results. Collection of data from patients treated with different types of antipsychotics

(both typical and atypical antipsychotics), as well as patients with combined antipsychotic treatment, is needed to obtain a more exact value for the remission rate. Finally, we did not examine the pathway of ADMA/NOS/NO in the present study. NOS activity and nitrate and nitrite levels should be measured to verify that there is a corresponding change in NO levels.

In conclusion, our present study involved a prospective, open-label, 8-week observational trial to explore the effects of atypical antipsychotics on cognitive function and plasma ADMA levels in patients with schizophrenia. The results showed that atypical antipsychotic treatment in schizophrenia patients with acute exacerbation improved psychiatric symptoms and cognitive function, particularly working memory and attention, in parallel with decreased plasma ADMA levels. The change in ADMA was negatively correlated with the improvement of cognition, suggesting that plasma ADMA may be a potential indicator of cognitive recovery in schizophrenia.

AUTHOR CONTRIBUTIONS

ZY, YZ, JZ, TL, JX, and BY were responsible for the clinical data collection and laboratory experiments. YY and BW were responsible for the study design, statistical analysis, and manuscript preparation. All authors have contributed to and approved the final manuscript.

FUNDING

This work was funded by the National Natural Science Foundation of China (No. 81560232, 81600939, and 81760254) and the Natural Science Foundation of Jiangxi Province of China (No. 20161BAB205193 and 20171BAB205020).

ACKNOWLEDGMENTS

We are deeply grateful to all participants.

REFERENCES

1. Brewer WJ, Wood SJ, Phillips LJ, Francey SM, Pantelis C, Yung AR, et al. Generalized and specific cognitive performance in clinical high-risk cohorts: a review highlighting potential vulnerability markers for psychosis. *Schizophr Bull.* (2006) 32:538–55. doi: 10.1016/S0920-9964(06)70139-6
2. Nuechterlein KH, Ventura J, Subotnik KL, Bartzokis G. The early longitudinal course of cognitive deficits in schizophrenia. *J Clin Psychiatry* (2014) 75 (Suppl. 2):25–9. doi: 10.4088/JCP.13065su1.06
3. Williams LM, Whitford TJ, Flynn G, Wong W, Liddell BJ, Silverstein S, et al. General and social cognition in first episode schizophrenia: identification of separable factors and prediction of functional outcome using the IntegNeuro test battery. *Schizophr Res.* (2008) 99:182–91. doi: 10.1016/j.schres.2007.10.019
4. Palmer BW, Dawes SE, Heaton RK. What do we know about neuropsychological aspects of schizophrenia? *Neuropsychol Rev.* (2009) 19:365–84. doi: 10.1007/s11065-009-9109-y
5. Green MF. Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *J Clin Psychiatry* (2006) 67 (Suppl. 9):3–8; discussion 36–42. doi: 10.4088/JCP.1006e12
6. Carrion RE, Goldberg TE, Mclaughlin D, Auther AM, Correll CU, Cornblatt BA. Impact of neurocognition on social and role functioning in individuals at clinical high risk for psychosis. *Am J Psychiatry* (2011) 168:806–13. doi: 10.1176/appi.ajp.2011.10081209
7. Lally J, Maccabe JH. Antipsychotic medication in schizophrenia: a review. *Br Med Bull.* (2015) 114:169–79. doi: 10.1093/bmb/ldv017
8. Tandon R. Antipsychotics in the treatment of schizophrenia: an overview. *J Clin Psychiatry* (2011) 72 (Suppl. 1):4–8. doi: 10.4088/JCP.10075su1.01
9. Gallhofer B, Bauer U, Lis S, Krieger S, Gruppe H. Cognitive dysfunction in schizophrenia: comparison of treatment with atypical antipsychotic agents and conventional neuroleptic drugs. *Eur Neuropsychopharmacol.* (1996) 6 (Suppl. 2):S13–20. doi: 10.1016/0924-977X(96)00010-7
10. Bilder RM, Goldman RS, Volavka J, Czobor P, Hoptman M, Sheitman B, et al. Neurocognitive effects of clozapine, olanzapine, risperidone, and haloperidol in patients with chronic schizophrenia or schizoaffective disorder. *Am J Psychiatry* (2002) 159:1018–28. doi: 10.1176/appi.ajp.159.6.1018
11. Krakowski MI, Czobor P, Nolan KA. Atypical antipsychotics, neurocognitive deficits, and aggression in schizophrenic patients. *J Clin Psychopharmacol.* (2008) 28:485–93. doi: 10.1097/JCP.0b013e3181855cd6
12. Wang J, Hu M, Guo X, Wu R, Li L, Zhao J. Cognitive effects of atypical antipsychotic drugs in first-episode drug-naive schizophrenic patients. *Neural Regen Res.* (2013) 8:277–86.doi: 10.3969/j.issn.1673-5374.2013.03.011
13. Asif M, Soiza RL, Mcevoy M, Mangoni AA. Asymmetric dimethylarginine: a possible link between vascular disease and dementia. *Curr Alzheimer Res.* (2013) 10:347–56. doi: 10.2174/1567205011310040001
14. Das I, Khan NS, Puri BK, Hirsch SR. Elevated endogenous nitric oxide synthase inhibitor in schizophrenic plasma may reflect abnormalities in brain nitric oxide production. *Neurosci Lett.* (1996) 215:209–11. doi: 10.1016/0304-3940(96)12972-4
15. Celik C, Cayci T, Ozdemir B, Akgul EO, Zincir S, Balikci A, et al. Plasma asymmetric dimethylarginine (ADMA) concentrations in patients with first and multiple episode schizophrenia. *Psychiatry Res.* (2011) 190:177–80. doi: 10.1016/j.psychres.2011.06.003
16. Zincir S, Zincir SB, Doruk A, Erdem M, Celik C, Ak M, et al. Asymmetric dimethylarginine (ADMA) and treatment response relationship in male patients with first-episode schizophrenia: a controlled study. *Psychiatry Res.* (2014) 220:76–80. doi: 10.1016/j.psychres.2014.07.013
17. Yang YJ, Xiong JW, Zhao Y, Zhan JQ, Chen HB, Yan K, et al. Increased plasma asymmetric dimethylarginine is associated with cognitive deficits in patients with schizophrenia. *Psychiatry Res.* (2016) 246:480–4. doi: 10.1016/j.psychres.2016.10.015
18. Palomo I, Contreras A, Alarcon LM, Leiva E, Guzman L, Mujica V, et al. Elevated concentration of asymmetric dimethylarginine (ADMA) in individuals with metabolic syndrome. *Nitric Oxide* (2011) 24:224–8. doi: 10.1016/j.niox.2011.03.002
19. Scott JA, North ML, Rafii M, Huang H, Pencharz P, Subbarao P, et al. Asymmetric dimethylarginine is increased in asthma. *Am J Respir Crit Care Med.* (2011) 184:779–85. doi: 10.1164/rccm.201011-1810OC
20. Chen GF, Moningka NC, Sasser JM, Zharikov S, Cunningham M Jr, Tain YL, et al. Arginine and asymmetric dimethylarginine in puromycin aminonucleoside-induced chronic kidney disease in the rat. *Am J Nephrol.* (2012) 35:40–8. doi: 10.1159/000334740
21. Kurtoglu E, Balta S, Sincer I, Altas Y, Atas H, Yilmaz M, et al. Comparision of effects of rosuvastatin versus atorvastatin treatment on plasma levels of asymmetric dimethylarginine in patients with hyperlipidemia having coronary artery disease. *Angiology* (2014) 65:788–93. doi: 10.1177/0003319713507333
22. Bekpinar S, Vardagli D, Unlucerci Y, Can A, Uysal M, Gurdol F. Effect of rosiglitazone on asymmetric dimethylarginine metabolism in thioacetamide-induced acute liver injury. *Pathophysiology* (2015) 22:153–7. doi: 10.1016/j.pathophys.2015.06.003
23. Karakoc A, Sahin A, Polat ES, Aliyev E, Yildirim A, Bakan N, et al. Serum apelin and ADMA levels in type 2 diabetics with and without vascular complications. *Diabetes Metab Syndr.* (2016) 10:S106–9. doi: 10.1016/j.dsx.2016.03.005

24. Guo X, Li J, Wang J, Fan X, Hu M, Shen Y, et al. Hippocampal and orbital inferior frontal gray matter volume abnormalities and cognitive deficit in treatment-naive, first-episode patients with schizophrenia. *Schizophr Res.* (2014) 152:339–43. doi: 10.1016/j.schres.2013.12.015
25. Xiong JW, Wei B, Li YK, Zhan JQ, Jiang SZ, Chen HB, et al. Decreased plasma levels of gasotransmitter hydrogen sulfide in patients with schizophrenia: correlation with psychopathology and cognition. *Psychopharmacology* (2018) 235:2267–74. doi: 10.1007/s00213-018-4923-7
26. Andreasen NC, Carpenter WT Jr, Kane JM, Lasser RA, Marder SR, Weinberger DR. Remission in schizophrenia: proposed criteria and rationale for consensus. *Am J Psychiatry* (2005) 162:441–9. doi: 10.1176/appi.ajp.162.3.441
27. Zhang C, Fang Y, Xu L. Glutamate receptor 1 phosphorylation at serine 845 contributes to the therapeutic effect of olanzapine on schizophrenia-like cognitive impairments. *Schizophr Res.* (2014) 159:376–84. doi: 10.1016/j.schres.2014.07.054
28. Deiana S, Watanabe A, Yamasaki Y, Amada N, Kikuchi T, Stott C, et al. MK-801-induced deficits in social recognition in rats: reversal by aripiprazole, but not olanzapine, risperidone, or cannabidiol. *Behav Pharmacol.* (2015) 26:748–65. doi: 10.1097/FBP.0000000000000178
29. Chen G, Lin X, Li G, Jiang D, Lib Z, Jiang R, et al. Risperidone reverses the spatial object recognition impairment and hippocampal BDNF-TrkB signalling system alterations induced by acute MK-801 treatment. *Biomed Rep.* (2017) 6:285–90. doi: 10.3892/br.2017.850
30. Yanik M, Vural H, Kocyigit A, Tutkun H, Zoroglu SS, Herken H, et al. Is the arginine-nitric oxide pathway involved in the pathogenesis of schizophrenia? *Neuropsychobiology* (2003) 47:61–5. doi: 10.1159/000070010
31. Pitsikas N. The role of nitric oxide donors in schizophrenia: basic studies and clinical applications. *Eur J Pharmacol.* (2015) 766:106–13. doi: 10.1016/j.ejphar.2015.09.045
32. Reif A, Herterich S, Strobel A, Ehlis AC, Saur D, Jacob CP, et al. A neuronal nitric oxide synthase (NOS-I) haplotype associated with schizophrenia modifies prefrontal cortex function. *Mol Psychiatry* (2006) 11:286–300. doi: 10.1038/sj.mp.4001779
33. Lauer M, Johannes S, Fritzen S, Senitz D, Riederer P, Reif A. Morphological abnormalities in nitric-oxide-synthase-positive striatal interneurons of schizophrenic patients. *Neuropsychobiology* (2005) 52:111–7. doi: 10.1159/000087555
34. Lee BH, Kim YK. Reduced plasma nitric oxide metabolites before and after antipsychotic treatment in patients with schizophrenia compared to controls. *Schizophr Res.* (2008) 104:36–43. doi: 10.1016/j.schres.2008.06.005
35. Medina JH, Izquierdo I. Retrograde messengers, long-term potentiation and memory. *Brain Res Brain Res Rev.* (1995) 21:185–94. doi: 10.1016/0165-0173(95)00013-5
36. Susswein AJ, Katzoff A, Miller N, Hurwitz I. Nitric oxide and memory. *Neuroscientist* (2004) 10:153–62. doi: 10.1177/1073858403261226
37. Bohme GA, Bon C, Lemaire M, Reibaud M, Piot O, Stutzmann JM, et al. Altered synaptic plasticity and memory formation in nitric oxide synthase inhibitor-treated rats. *Proc Natl Acad Sci USA.* (1993) 90:9191–4. doi: 10.1073/pnas.90.19.9191
38. Ohno M, Yamamoto T, Watanabe S. Deficits in working memory following inhibition of hippocampal nitric oxide synthesis in the rat. *Brain Res.* (1993) 632:36–40. doi: 10.1016/0006-8993(93)91135-F
39. Fin C, Da Cunha C, Bromberg E, Schmitz PK, Bianchin M, Medina JH, et al. Experiments suggesting a role for nitric oxide in the hippocampus in memory processes. *Neurobiol Learn Mem.* (1995) 63:113–5. doi: 10.1006/nlme.1995.1011
40. Yamada K, Noda Y, Hasegawa T, Komori Y, Nikai T, Sugihara H, et al. The role of nitric oxide in dizocilpine-induced impairment of spontaneous alternation behavior in mice. *J Pharmacol Exp Ther.* (1996) 276:460–6. doi: 10.1016/0306-4522(96)00161-3
41. Beninger RJ, Forsyth JK, Van Adel M, Reynolds JN, Boegman RJ, Jhamandas K. Subchronic MK-801 behavioural deficits in rats: partial reversal by the novel nitrate GT 1061. *Pharmacol Biochem Behav.* (2009) 91:495–502. doi: 10.1016/j.pbb.2008.09.003
42. Issy AC, Pedrazzi JF, Yoneyama BH, Del-Bel EA. Critical role of nitric oxide in the modulation of prepulse inhibition in Swiss mice. *Psychopharmacology* (2014) 231:663–72. doi: 10.1007/s00213-013-3277-4
43. Alaqeel B, Margolese HC. Remission in schizophrenia: critical and systematic review. *Harv Rev Psychiatry* (2012) 20:281–97. doi: 10.3109/10673229.2012.747804

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 24 January 2019
doi: 10.3389/fpsyt.2018.00767

Glutamatergic Dysfunction and Glutamatergic Compounds for Major Psychiatric Disorders: Evidence From Clinical Neuroimaging Studies

Cheng-Ta Li[1,2,3,4]*, *Kai-Chun Yang*[1,3] *and Wei-Chen Lin*[1,3]

[1] Department of Psychiatry, Taipei Veterans General Hospital, Taipei, Taiwan, [2] Institute of Brain Science and Brain Research Center, National Yang-Ming University, Taipei, Taiwan, [3] Division of Psychiatry, Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan, [4] Institute of Cognitive Neuroscience, National Central University, Jhongli, Taiwan

Excessive glutamate release has been linked to stress and many neurodegenerative diseases. Evidence indicates abnormalities of glutamatergic neurotransmission or glutamatergic dysfunction as playing an important role in the development of many major psychiatric disorders (e.g., schizophrenia, bipolar disorder, and major depressive disorder). Recently, ketamine, an *N*-methyl-d-aspartate antagonist, has been demonstrated to have promisingly rapid antidepressant efficacy for treatment-resistant depression. Many compounds that target the glutamate system have also become available that possess potential in the treatment of major psychiatric disorders. In this review, we update evidence from recent human studies that directly or indirectly measured glutamatergic neurotransmission and function in major psychiatric disorders using modalities such as magnetic resonance spectroscopy, positron emission tomography/single-photon emission computed tomography, and paired-pulse transcranial magnetic stimulation. The newer generation of antidepressants that target the glutamatergic system developed in human clinical studies is also reviewed.

Keywords: glutamate, major psychiatric disorders, NMDA antagonist, antidepressant, neuroimaging

OPEN ACCESS

Edited by:
Hsien-Yuan Lane,
China Medical University, Taiwan

Reviewed by:
Shigeyuki Chaki,
Taisho Pharmaceutical, Japan
Chun Yang,
Huazhong University of Science and Technology, China

***Correspondence:**
Cheng-Ta Li
ctil2@vghtpe.gov.tw

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *03 September 2018*
Accepted: *21 December 2018*
Published: *24 January 2019*

Citation:
Li C-T, Yang K-C and Lin W-C (2019) Glutamatergic Dysfunction and Glutamatergic Compounds for Major Psychiatric Disorders: Evidence From Clinical Neuroimaging Studies. Front. Psychiatry 9:767. doi: 10.3389/fpsyt.2018.00767

INTRODUCTION

Overview of Glutamate and Risks of Neuropsychiatric Disorders

Glutamate is the most abundant excitatory neurotransmitter in the human brain and has critical roles in multiple brain functions and synaptic plasticity, such as long-term potentiation. However, excessive glutamate release can be toxic to the brain and has been linked to many neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease (1). Glutamate excitotoxicity has been associated with exposure to severe stress, and excessive glutamate release and uptake have been identified in brain regions such as the frontal cortex and hippocampus of rats exposed to various forms of stress (2, 3). A growing body of evidence also indicates that abnormalities of glutamatergic neurotransmission play an important role in the development of many major psychiatric disorders (e.g., schizophrenia, bipolar disorder [BD], and major depressive disorder [MDD], including treatment-resistant depression [TRD]) (4, 5). In this review, we first update evidence from recent neuroimaging human studies that pinpoints glutamatergic dysfunction in the pathophysiology of major psychiatric disorders.

Glutamate Receptors and Subunits

Glutamate receptors can be divided into two categories: ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) (6). iGluRs with an ion channel pore activate when glutamate binds to receptors, whereas mGluRs activate ion channels on the plasma membrane indirectly through a signaling cascade. iGluRs have three subtypes of receptors—*N*-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and kainate receptors—based on the chemical that binds to them more selectively than glutamate. Mammalian mGluRs are categorized into three groups: group 1 (mGluR1 and mGluR5), group 2 (mGluR2 and mGluR3), and group 3 (mGluR4, mGluR6, mGluR7, and mGluR8). Activation of the NMDA receptor (NMDAR) requires a glutamate binding to its NR2 subunits and a glycine binding to its NR1 subunits. Then, a nonspecific cation channel is opened, enabling Ca^{2+} and Na^{+} to enter and K^{+} to exit the cell (7).

Neuroimaging Techniques in Assessing Glutamate-Related Function in Human Brains

To date, it remains difficult to measure glutamatergic neurotransmission in human brains. However, some techniques have been developed to explore glutamatergic neurotransmission by calculating glutamate levels, such as magnetic resonance spectroscopy (MRS), or by measuring glutamate-related function, including positron emission tomography (PET)/single-photon emission computed tomography (SPECT) and paired-pulse transcranial magnetic stimulation (ppTMS).

MRS is a specialized technique that directly quantifies brain molecules, including glutamate. This noninvasive and ionizing radiation-free neuroimaging technique is associated with magnetic resonance imaging (MRI), and both are used to acquire signal from hydrogen protons (or other nuclei, including carbon and nitrogen).

Typically, MRS is used to measure signals within a predefined region of interest (i.e., a voxel), although the technique is still being updated to acquire biochemical signals from the whole brain (8). Using ^{1}H-MRS, and depending on the chemical environment, each proton may be visualized at a specific chemical shift (peak position along the chemical shift axis). Glutamate (Glu) and glutamine can thus be marked by a series of resonance peaks between 2.2 and 2.4 ppm, which means that these metabolites overlap and are often referred to in combination as Glx. Some editing techniques (e.g., echo time averaging) enable differentiation of glutamate from glutamine (9). It has been reported that field strengths of 4 T or more are better for the separation of glutamate from glutamine (10). However, the information of glutamate derived by MRS does not exactly reflect glutamatergic neurotransmission of the neurons but rather the total level/concentration in the given voxel.

PET enables measurement of brain molecules by detecting gamma rays originating from the annihilation between electrons and positrons emitting from radionuclides. iGluRs are ligand-gated ion channels that mediate excitatory neurotransmission in human brains and can be divided into at least three groups: NMDA, AMPA, and kainate receptors (11). Several radioligands have been developed to successfully image different subtypes of NMDARs *in vivo* (11). Moreover, human studies have been conducted using PET imaging with ^{11}C-ABP688, a radioligand for mGluR5, to evaluate ketamine-induced glutamate release both in healthy subjects (12) and in patients with MDD (13). However, the application of this PET imaging paradigm to measure glutamate receptors in clinical settings might be limited by the availability of the radioligand and the need for arterial input function for quantification. In addition, because PET/SPECT measures specific molecules, the findings do not represent exact glutamatergic neurotransmission. Alternatively, ^{18}F-fluorodeoxyglucose (^{18}F-FDG)-PET, a clinical imaging tool widely used to measure brain glucose uptake with favorable signal-to-noise ratio in most brain regions, has been proposed to be a proxy measure of glutamatergic neurotransmission (14, 15). The rationale is that glutamate is produced in neurons from glucose-derived tricarboxylic acid cycle intermediates and branched-chain amino acids. The reuptake of glutamate from the synaptic cleft is coupled with Na^{+}/K^{+}-ATPase activation and glucose use (16). With neuronal depolarization and glutamate being released into the synaptic cleft from presynaptic vesicles, the process requires energy and is dependent on the use of glucose. Interestingly, ketamine-induced increased glucose uptake in patients with MDD (14) is in line with decreased ^{11}C-ABP688 binding in similar brain regions (13). Thus, ^{18}F-FDG-PET might serve as a promising tool to evaluate glutamatergic neurotransmission.

ppTMS is a noninvasive technique that manipulates the strength and stimulus intervals between two pulses to measure cortical inhibition and excitation in humans (17, 18). It can be used to examine at least two different corticocortical inhibitory processes in the human motor cortex that are mediated by different subtypes of GABAergic receptors: short-interval cortical inhibition and long-interval cortical inhibition (19). Moreover, ppTMS can also be used to examine a corticocortical excitatory process, intracortical facilitation (ICF), when a subthreshold pulse precedes a test pulse by 8–30 ms (17, 20). The resulting facilitation of the motor-evoked potential response has been found to be mediated mainly by glutamatergic neurotransmission. When a glutamate antagonist, riluzole, is used, ICF can be suppressed without influencing cortical inhibition (21). Such findings indicate that the neurotransmitter glutamate is involved in facilitatory mechanisms of the motor cortex. Compared with the aforementioned techniques, ppTMS measurement such as ICF is more likely to reflect functional glutamatergic neurotransmission in the testing cortical region but not levels of the subtypes of glutamate receptors. In addition, I-wave facilitation is another ppTMS measurement that reflects glutamatergic activity of a different neuron population to ICF and could be mediated by non-NMDA receptors (22).

Schizophrenia and Glutamatergic Dysfunction

Schizophrenia is a major psychiatric disorder characterized by prominent psychotic symptoms and abnormal social behaviors. Despite most current antipsychotics being dopamine antagonists or acting on dopamine receptors, alterations

TABLE 1 | Summary of major neuroimaging findings to support glutamatergic dysfunction in major psychiatric disorders.

	Schizophrenia	Bipolar disorder	MDD
MRS	↑Glutamate (BG*, ACC, THA)	↑Glx (PFC*)	↓Glx (PFC*, ACC*)
	↑Glutamine (THA*, PFC, ACC)	Glutamate: no change*	↓Glutamate (ACC*)
	↑Glx (BG*, MTL*, PFC, OC, PC)		
PET	↓NMDA receptor binding (HIPPO)	Lack of direct evidence	↓mGluR5 (ACC, OFC, BG, AMG, HIPPO)
	↑Dopamine uptakes (BG)	↑Glucose uptakes (BG) after ketamine	↑Glucose uptakes (PFC) after ketamine
	↑Glucose uptakes and blood flow (ACC) after ketamine		
	↑Glucose uptakes (PFC) after ketamine		
pp-TMS	↑ICF (first-episode patients)	No reports on ICF and I-wave facilitation	ICF: no difference; but
	↑I-wave facilitation		↑ICF in young MDD

BG, Basal ganglia;ACC, anterior cingulate cortex; MTL, medial temporal lobe; THA, thalamus; PFC, prefrontal cortex; OFC, orbital frontal cortex; HIPPO, hippocampus; AMG, amygdala; PC, parietal cortex;OC, occipital cortex.

**Supported by at least one meta-analysis.*

in glutamatergic neurotransmission could be critical to the pathophysiology of schizophrenia. For example, administration of the NMDAR antagonist phencyclidine or ketamine could induce a schizophrenia-like state in human subjects (23, 24), supporting the hypothesis that glutamatergic dysfunction plays a crucial role in the pathophysiology of schizophrenia. Furthermore, group I mGluRs are heavily expressed in basal ganglia that contain high densities of dopamine receptors (25), and at least two independent studies have identified several deleterious single-nucleotide polymorphisms (SNPs) in the human gene encoding mGluR subtype I in patients with schizophrenia (26). Despite inconsistency, some postmortem studies have also revealed that iGluRs and mGluRs are abnormally expressed in human subjects with schizophrenia. For example, iGluR-AMPA receptors and kainate receptors were decreased in expression in the schizophrenic hippocampus, and the iGluR-NMDAR subunit NR1 might be abnormally expressed in some cortical regions in schizophrenia (27), whereas higher mRNA levels for group I mGluRs were found in the prefrontal cortex (Brodmann area 9) in patients with schizophrenia (28).

A large meta-analysis of ^{1}H-MRS studies identified 59 studies that included 1,686 patients and 1,451 healthy control subjects (**Table 1**) (29). By adopting a random-effects, inverse-weighted variance model to calculate the pooled effect size, the investigators found that, in schizophrenia, there were significant elevations in glutamate in the basal ganglia (Hedges' g = 0.63; 95% confidence interval [CI], 0.15–1.11), glutamine in the thalamus (Hedges' g = 0.56; 95% CI, 0.02–1.09), and Glx in the basal ganglia (Hedges' g = 0.39; 95% CI, 0.09–0.70) and the medial temporal lobe (Hedges' g = 0.32; 95% CI, 0.12–0.52). No regions exhibited a reduction in glutamate metabolites in schizophrenia. A systemic review pinpoints that increased Glx in many cortical regions, including prefrontal cortex, temporal cortex, parietal cortex, and occipital cortex, as well as increased glutamine in thalamus, prefrontal cortex, and anterior cingulate cortex (30). By contrast, a recent systemic review and meta-analysis of ^{1}H-MRS studies on antipsychotic-naïve/free patients with schizophrenia included 21 studies and noted no changes in glutamate-related metabolites (31). Because brain glutamate levels may be confounded by drug use and related to response to antipsychotics, Egerton et al. investigated glutamate levels (Glu/Cr) in the anterior cingulate cortex and thalamus in antipsychotic-naïve or minimally medicated patients with first-episode psychosis, and they found that higher levels of glutamate in the anterior cingulate cortex were associated with more severe psychotic symptoms at presentation and a lower likelihood of being in remission 4 weeks after amisulpride treatment (32).

Development of the glutamate hypothesis of schizophrenia was initially based on the effects of phencyclidine, which acts primarily as an NMDAR antagonist. Because presynaptic dopamine release is under the control of inhibitory GABAergic neurons that are activated by NMDARs, previous PET/SPECT studies that used D2/D3 receptor ligands (e.g., ^{11}C-raclopride and ^{123}I-iodobenzamide) in schizophrenia provided evidence for glutamate dysfunction in schizophrenia. A review study including D2/D3 receptor PET/SPECT studies revealed that increased dopamine uptake in the striatum and putamen was observed in schizophrenia, indicating an effect of NMDA blockade on striatal dopamine release (33). Not only findings in the basal ganglia, early studies measured cerebral blood flow following ketamine infusion in patients with schizophrenia by 15O-H2O PET and found increased blood flow in anterior cingulate cortex (34), which was found to correlate the ketamine-induced psychosis-effects in healthy control subjects (35). A study using 18F-FDG PET to study glucose metabolism after ketamine also revealed increased metabolism in frontal cortex and anterior cingulate cortex (36). In addition, repeated ketamine administration had been found to have increased dopamine D1 receptor binding in the dorsolateral prefrontal cortex by using 11C-NNC112 (37). As for studies using radioligands to image glutamatergic receptors directly, 123I-CNS-1261 as a SPECT ligand acts on NMDA receptors had been used in healthy subjects to study the binding of ketamine and the results found ketamine led to a global reduction in the binding signals (38). One study found the reduction of 123I-CNS-1261 binding after ketamine was greatest in thalamus, basal ganglia, and frontal cortex, which mainly correlated with negative symptoms (39). While marked reduction of NMDA receptor bindings had been reported in

patients treated with schizophrenia, a study directly applying this compound in medication-free patients with schizophrenia found significant reductions of NMDA receptor binding in left hippocampus (40).

ICF of ppTMS reflects glutamatergic neurotransmission in the motor cortex. A meta-analysis including ppTMS studies from 1990 to 2012 found no changes of ICF, but decreased short-interval intracortical inhibition, in schizophrenia (41). By contrast, later research with small sample sizes revealed significant increases of ICF in first-episode schizophrenia compared with healthy control subjects (42). In addition, I-wave facilitation was found to be increased in patients with schizophrenia (43). Future studies with larger sample sizes controlling chronicity of illness course and the use of medications are still needed.

Bipolar Disorder and Glutamatergic Dysfunction

BD is a major psychiatric disorder characterized by prominent mood fluctuation, including episodes of depression and periods of abnormally elevated mood. Postmortem studies of patients with BD revealed reduced expression of NMDAR subunit NR1 in the prefrontal cortex (44) and reduced expression of several NMDA, AMPA, and kainite receptor subunits in the medial temporal cortex (45), although other postmortem research found that mGluRs seem to be less involved in the anterior cingulum of patients with BD (46). However, genome-wide association studies (GWAS) and SNP results have also provided genetic evidence that glutamate signaling is implicated in the pathophysiology of BD (47, 48).

Previous neuroimaging studies in patients with BD (**Table 1**) have provided evidence that glutamate dysfunction plays a crucial role in the pathophysiology of BD. A meta-analysis of ^{1}H-MRS studies from 1980 to 2010 on brain glutamate and glutamine in BD showed that patients with BD had widespread increased Glx, including in the prefrontal cortex, compared with healthy control subjects, although no significant difference in Glu/Cr was noted (49). The finding of increased Glx in the frontal cortex was replicated in a subsequent meta-analysis (50). However, the results to date have been inconsistent, and many factors, including mood status and medication, might affect glutamate levels. For example, a recent systemic review examined the effects of lithium, a drug commonly used for mood control, by summarizing results of 26 ^{1}H-MRS studies (51). The investigators found inconclusive results regarding glutamate levels and the influence of lithium treatment.

Few PET/SPECT studies have used radioligands specifically to target glutamate receptors in BD. However, ketamine, an NMDA antagonist, has been found to have rapid antidepressant effects in depressed patients with BD and has been studied in combination with ^{18}F-FDG-PET before and after ketamine infusion. That study found that brain glucose metabolism changes in the right ventral striatum of basal ganglia were significantly correlated with depression improvement (52). Because ^{18}F-FDG PET in combination with glutamatergic agents could serve as a proxy for glutamate neurotransmission, the findings suggest that ketamine improved BD depression by promoting glutamatergic neurotransmission in brain regions involved in mood control. However, questions remain. For example, whether glutamate dysregulation is the fundamental cause of the pathophysiology of BD and whether glutamatergic agents can reverse the brain abnormalities of BD. We compared brain glucose metabolism in subtypes of BD by using ^{18}F-FDG-PET and found that patients with BD type I (compared with patients with BD type II) had significantly lower glucose metabolism in the bilateral anterior cingulum, insula, striatum, and part of the prefrontal cortex and higher glucose metabolism in some limbic structures (53). Frontolimbic dysregulation seems to play a critical role in the pathophysiology of BD, because the unaffected siblings of patients with BD have such abnormalities in a minor form that can be detected by resting-state functional MRI but not by ^{18}F-FDG-PET (54). Whether these abnormal findings are a result of glutamate dysregulation warrants further investigation. Furthermore, ppTMS research that specifically examines ICF is still lacking, despite such research demonstrating cortical inhibitory deficits in patients with BD (55).

Major Depressive Disorder/Treatment-Resistant Depression and Glutamatergic Dysfunction

MDD is a severe psychiatric disorder characterized by episodes of depression and anhedonia. MDD is considered a severe illness because of a tendency for the illness to become chronic and a high prevalence of TRD. Evidence has revealed a pivotal role of glutamatergic neurotransmission in the pathophysiology of MDD.

Postmortem studies have revealed that expression of mGluR2/3 receptors in the anterior cingulate cortex was significantly reduced in patients with MDD (56), whereas another study found no significant difference in the anterior cingulate cortex between patients with MDD and healthy control subjects in expression of mGluR2/3 or mGluR5 (46). Furthermore, iGluRs are abnormally expressed in human subjects with MDD. For example, the expression of NMDAR subunit NR2 in the prefrontal cortex was reduced in patients with MDD (57). A study using postmortem brains of patients with MDD further demonstrated that elevated expression levels of the majority of mGluR and iGluR genes were found in the dorsolateral prefrontal cortex, and the genetic expression differences occurred mostly in female subjects (58). A meta-analysis of three large MDD GWASs (4,346 subjects with MDD vs. 4,430 control subjects) found that genes involved in glutamatergic synaptic neurotransmission were significantly associated with MDD (59).

Regarding neuroimaging findings in clinical patients (**Table 1**), ^{1}H-MRS studies in patients with MDD have revealed decreased glutamate and glutamine levels in the dorsolateral and other parts of the prefrontal cortex and increased glutamate levels in the occipital cortex (60). A meta-analysis of ^{1}H-MRS studies demonstrated that decreased Glx levels with absolute values in the prefrontal cortex were correlated with treatment severity (i.e., number of failed antidepressant treatments),

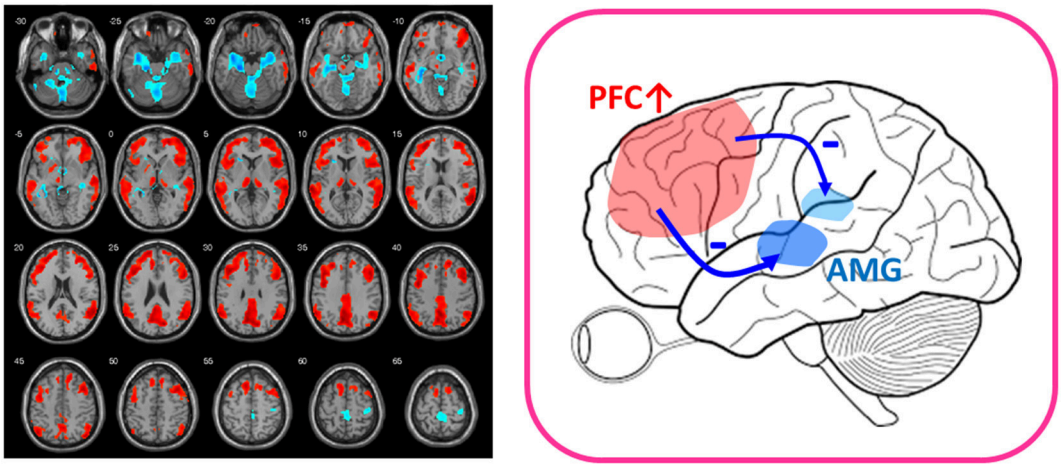

FIGURE 1 | After-vs.-before changes of glucose metabolism in response to low-dose ketamine in treatment-resistant depressives (FWE-corrected, $p < 0.001$). Patients showed decreased glucose metabolism in limbic structures, such as amygdala (AMG) and hippocampus (shown in blue color) and increased function in prefrontal cortex (PFC) (shown in red color) after ketamine treatment. The placebo group lacked the PFC activation (14). The findings of prefronto-amygdala changes in response to ketamine provided supports that low-dose ketamine could reverse glutamatergic dysfunction of the mood circuit.

indicating that the severity of glutamatergic dysregulation could be related to the severity of illness (61). Another meta-analysis noted that glutamate and Glx concentrations were lower in the anterior cingulate cortex in patients with MDD than in control subjects (62).

A further piece of evidence comes from the surprisingly rapid antidepressant response to low-dose ketamine in the treatment of TRD. Structural and functional abnormalities in the prefrontal cortex have been found to be prominent in patients with TRD (63, 64), and intravenous low-dose ketamine (0.2–0.5 mg/kg) was revealed to reverse the prefrontal abnormalities and frontolimbic dysregulation of the human brain in 1 h (14). We applied ^{18}F-FDG-PET before and after intravenous injection of 0.5 mg/kg ketamine, 0.2 mg/kg ketamine, and placebo and found that prefrontal cortical function increased only in the low-dose ketamine groups, whereas the activation of prefrontal function correlated well with the deactivation of limbic function in the amygdala and hippocampus (**Figure 1**) (14). Moreover, recent PET research using ^{11}C-ABP688, a radioligand for mGluR5, revealed a significant ketamine-induced reduction in mGluR5 availability as reflected by decreased ^{11}C-ABP688 binding in all subjects, which persisted for more than 24 h (13). In addition, the changes of ^{11}C-ABP688 binding were correlated with the rapid antidepressant effect of ketamine. However, because ketamine is an NMDA antagonist (one type of iGluR), future studies directly investigating iGluRs in response to low-dose ketamine are warranted.

Although some ppTMS studies have found no significant differences in ICF between patients with MDD and healthy subjects (41, 65), few studies have differentiated MDD from TRD. In addition, medication could obviously confound the ppTMS findings, given that medicated euthymic patients with MDD exhibited much increased ICF compared with healthy control subjects and unmedicated patients with MDD (65). Furthermore, patients with MDD with a comorbidity of generalized anxiety disorder are more likely to have poorer responses to antidepressants than those without (66). Our team demonstrated that patients with generalized anxiety disorder in unmedicated status had decreased ICF in bilateral motor cortices, suggesting that patients with more impaired glutamatergic neurotransmission in cortical regions may have worse treatment outcomes (5). A study targeting at child and adolescence depressives (9–17 years old) and found depressed patients had significantly increased ICF (67). Future studies that control medications and more specifically target TRD over MDD without a history of antidepressant resistance are necessary.

Glutamate Compounds That Target Glutamatergic Dysfunction

As aforementioned, glutamate receptors can be divided into iGluRs and mGluRs (6). iGluRs include NMDARs, AMPA receptors, and kainate receptors, whereas mGluRs can be categorized into group 1, group 2 (mGluR2 and mGluR3), and group 3. More recently, selective glutamate positive allosteric modulators (PAMs) have been developed that enhance glutamate receptor function in the presence of endogenous agonists without having adverse effects resulting from intrinsic activity (e.g., NMDAR PAMs) (68). By contrast, glutamate mGluR2 or mGluR5 (basimglurant) negative allosteric modulators (NAMs) may inhibit glutamate receptor function and have been tried in MDD studies, although the results are mixed (69). Therefore, given that glutamatergic dysfunction plays a crucial role in the major psychiatric disorders discussed herein, compounds aimed at activating or inhibiting the aforementioned receptors or indirectly modulating functions of receptors are of great research interest. **Table 2** lists glutamatergic compounds that have been

TABLE 2 | Glutamatergic compounds in the treatment of major psychiatric disorders.

Glutamate receptors	Compounds	Mechanisms	Target diseases	Examples of ClinicalTrials.gov Identifier (C: completed; T: terminated)
iGluR-NMDA	Ketamine	An NMDA antagonist	MDD in alcoholism	NCT01551329 (phase 1) (C)
			Bipolar depression	NCT01833897 (phase 4) (C)
	Esketamine	An NMDA antagonist	MDD (TRD)	NCT02782104 (phase 3)
			Imminent suicide risks	NCT02133001 (phase 2) (C)
	D-cycloserine	Mixed agonist/antagonist at NMDA receptor/glycine binding site	MDD (TRD)	NCT00408031 (phase 2) (C)
			Bipolar depression	NCT01833897 (phase 4) (C)
			Schizophrenia	NCT02769936 (phase 1) (C)
	D-serine	An NMDA-glycine site agonist	Schizophrenia	NCT00322023 (phase 2) (C)
	RO4917838 (Bitopertin)	A glycine reuptake inhibitor	Schizophrenia	NCT01235585 (phase 3) (C)
	NRX-101	D-cycloserine + lurasidone	Bipolar depression	NCT03395392 (phase 2)
	Riluzole	A glutamate release inhibitor	MDD (TRD)	NCT00088699 (phase 2) (C)
			Bipolar depression	NCT00054704 (phase 2) (T)
	Nitrous oxide	An NMDA antagonist	MDD (TRD)	NCT02994433 (phase 1)
	NMDAE	An NMDA enhancer	MDD	NCT03414931 (phase 2) (C)
	Nuedexta	Dextromethorphan+quinidine	MDD (TRD)	NCT01882829 (phase 2) (C)
		Dextromethorphan as an NMDA antagonist		
	AXS-05	Dextromethorphan+bupropion	MDD (TRD)	NCT02741791 (phase 3)
		Dextromethorphan as an NMDA antagonist		
	CP-101,606 (traxoprodil)	An NMDA receptor subunit GluN2B Antagonist	MDD (TRD)	NCT00163059 (phase 2) (C)
	Memantine	An NMDA antagonist	MDD	NCT00040261 (phase 3) (C)
	Sarcosine	An NMDA enhancing agent (a glycine transporter-I inhibitor)	MDD	NCT00977353 (phase 2) (C)
			Schizophrenia	NCT01503359 (phase 2) (C)
	AZD6765	An NMDA channel blocker	MDD (TRD)	NCT00986479 (phase 2) (C)
	CERC-301	An NMDA GluN2B antagonist	MDD	NCT02459236 (phase 2) (C)
	MK-0657	A selective NMDA GluN2B antagonist	MDD (TRD)	NCT00472576 (phase 2) (C)
	NRX-1074	An NMDA partial agonist	MDD	NCT02067793 (phase 2) (C)
	GLYX-13 (Rapastinel)	An NMDA receptor enhancer	MDD (TRD)	NCT01684163 (phase 2) (C)
	REL-1017 (d-Methadone)	A non-opioid NMDA receptor antagonist	MDD (TRD)	NCT03051256 (phase 2)
	EVT-101	An NMDA GluN2B antagonist	MDD (TRD)	NCT01128452 (phase 2) (T)
iGluR-AMPA	ORG 24448	an AMPAkine as AMPA receptor potentiators	MDD	NCT00262665 (withdrawn)
			Schizophrenia	NCT00425815 (withdrawn)
	CX516	An AMPA receptor positive modulator	Schizophrenia	NCT00235352 (phase 3) (C)
mGluR or other pathways	N-Acetyl-Cysteine (NAC)	May restore glutamate to its correct levels in the brain	Schizophrenia	NCT02505477 (phase 4)
			MDD (TRD)	NCT02972398
	Pomaglumetad methionil (LY2140023)	Metabotropic glutamate 2/3 receptor (mGluR2/3R) agonist	Schizophrenia	NCT00149292 (phase 2) (C)
			Schizophrenia	NCT01307800 (phase 3) (T)
	JNJ-40411813 (ADX-71149)	mGluR2 positive allosteric modulator	Schizophrenia	NCT01323205 (phase 2) (C)
	AZD-8529	mGluR2 positive allosteric modulator	Schizophrenia	NCT00921804 (phase 2) (C)
	RO4995819 (Decoglurant)	GluR2/3 negative allosteric modulator	MDD	NCT01733654 (withdrawn)
	Basimglurant	mGluR5 negative allosteric modulator	MDD	NCT01437657 (phase 2) (C)
	Diazoxide	Increases glutamate uptake from the synaptic cleft	MDD	NCT02049385 (phase 1) (T)
	Ceftriaxone	Decreasing the amount of extracellular glutamate in brain	Schizophrenia	NCT00591318 (phase 1) (T)

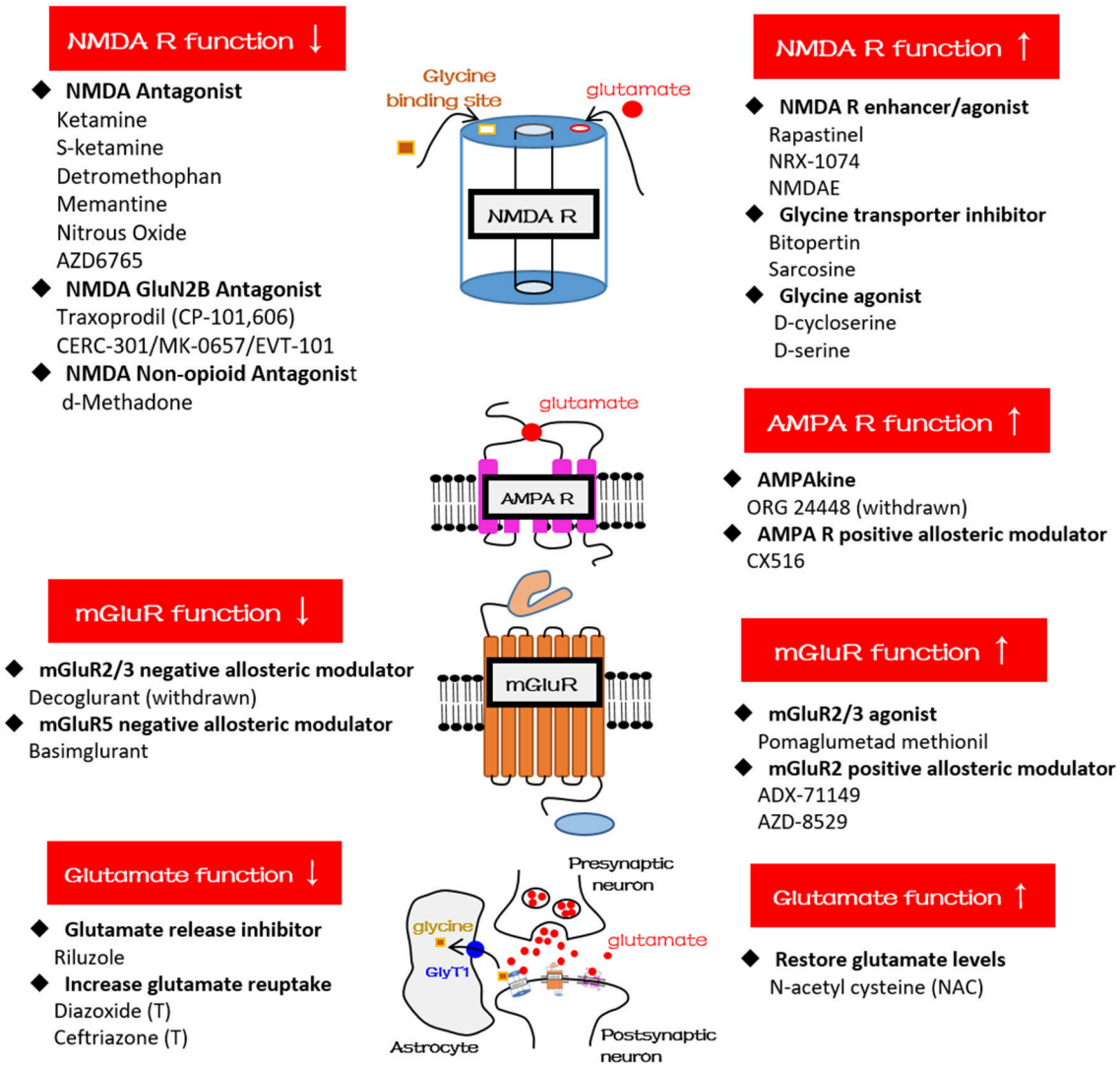

FIGURE 2 | Schematic diagrams and glutamatergic compounds for NMDA receptor, AMPA receptor, mGluR, and glutamatergic neurotransmission, respectively. GlyT1, glycine transporter type-1.

used to treat major psychiatric disorders in human clinical trials (searched in the ClinicalTrials.gov database, accessed on September 20, 2018).

The most attractive compounds are NMDA antagonists because a growing body of evidence has pinpointed glutamatergic dysfunction in the pathophysiology of TRD and demonstrated that the glutamatergic synapses present multiple targets for development of novel antidepressants. For example, ketamine and its S-enantiomer (esketamine) are NMDA antagonists and, when used in a low-dose range, have exhibited rapid antidepressant properties for TRD (70). In a low-dose range, tolerability seems to be acceptable with transient elevation of blood pressure and mild and self-limited psychotomimetic effects (70). Several other compounds, such as dextromethorphan, memantine, traxoprodil, AZD6765, and riluzole, among others (see **Table 2** and **Figure 2**), have similar pharmacological properties and have great potential in treating MDD and BD depression. Using a small sample size (n = 14 completed the study), researchers in an open-label study found that dextromethorphan/quinidine (Nuedexta; Avanir Pharmaceuticals, Inc., Aliso Viejo, CA, USA) could decrease depression scores with acceptable tolerability in patients with TRD (71). Traxoprodil, an NR2B subunit-selective NMDAR antagonist, in combination with paroxetine was found to decrease depression scores for patients with TRD (n = 30) (72). A single intravenous dose of AZD6765 (a low-trapping NMDA channel blocker) was also found to have rapid but short-lived antidepressant effects in a small trial (n = 22) (73). However, longer-duration and larger studies are required to prove clinical efficacy because not all NMDA antagonists possess rapid antidepressant efficacy. For example, an NMDA antagonist, memantine (74), had been shown to lack rapid antidepressant

effects. Likewise, riluzole was found to have limited effects for ketamine nonresponders (75). Notably, sarcosine, an NMDA enhancer, had been found to improve depression-like behavior in rodent models and depression in humans (76). Some iGluR NMDA-related compounds are also used for treating schizophrenia (**Table 2**). For example, D-serine, a naturally occurring NMDAR glycine site agonist, was found to have significant effects on auditory mismatch negativity that correlated significantly with change in symptoms of schizophrenia in a small double-blind crossover trial ($n = 16$) (77). Researchers in a randomized, multicenter, double-blind, placebo-controlled study investigated adjunctive RO4917838 (bitopertin), a selective GlyT1-mediated glycine reuptake inhibitor, in patients with schizophrenia with suboptimally controlled symptoms ($n = 1{,}772$) and found that the antipsychotic effects were small (mean difference vs. placebo in score, −1.37) and were demonstrated in only one of six active treatment arms (78). Some trials have been withdrawn or terminated (**Table 2**), including a trial using EVT-101 for MDD, trials using Org 24448 for MDD and schizophrenia, a trial using diazoxide for MDD, and a trial using ceftriaxone for schizophrenia. The reasons may include prominent side effects or a lack of clinical efficacy, so the final outcomes of these clinical trials must be awaited.

Glutamatergic compounds are also used for treating BD depression. For example, researchers in a randomized, double-blind, placebo-controlled study investigating a single intravenous infusion of ketamine (0.5 mg/kg) for BD depression found that depressive symptoms and suicidal ideation significantly improved within 40 min (79). In addition, a small proof-of-concept study investigated the effects of ketamine and D-cycloserine in patients with BD, in which subjects received open-label ketamine hydrochloride (0.5 mg/kg intravenously over 60 min) followed by 8 weeks of adjunctive D-cycloserine (titrated up to 1000 mg/d from a starting dose of 250 mg over 3 weeks) (80). The investigators found that four of seven subjects met remission criteria at 8 weeks. However, a randomized controlled study of riluzole monotherapy (50–200 mg/d) for BD depression was terminated early (**Table 2**) because of a high number of subject withdrawals and no significant antidepressant effects of riluzole (81).

Although most iGluR NMDA compounds seem to be used for treating MDD (**Table 2**), iGluR-AMPA and mGluR compounds are generally used for treating schizophrenia (**Table 2**). For example, CX516 (an AMPA receptor-positive modulator and also the first ampakine) was used for cognitive enhancement in schizophrenia; however, the results appear to be disappointing because CX516 was not effective for cognition or for symptoms of schizophrenia when added to clozapine, olanzapine, or risperidone (82). *N*-acetylcysteine is a widely available dietary supplement that may restore glutamate to its correct levels in the brain and is used for treating cognitive deficits in schizophrenia (**Table 2**). LY2140023 as an mGluR2/3 agonist was originally found to have significant antipsychotic effects in patients with schizophrenia early in disease or in those previously treated with D2 drugs (83), but investigators terminated another study because LY2140023 failed to achieve significant effects on the overall symptoms of schizophrenia (**Table 2**).

Hypofunction of NMDARs has been suggested to play an important role in the pathophysiology of schizophrenia, and glutamate PAMs may be effective for treating schizophrenia and related cognitive deficits. However, to date, the results have been inconsistent. For example, authors of a recent meta-analysis that included 17 randomized, placebo-controlled studies ($n = 1{,}391$) found that glutamate PAMs were not superior to placebo in improving cognitive function in schizophrenia (84). In addition, results of mGluR2 activators for schizophrenia seem to be inconsistent. For example, Eli Lilly's mGluR2/3 agonist (LY2140023) failed to meet the primary endpoints in a phase II trial (85), and another phase III trial was stopped mainly owing to the lack of efficacy; however, JNJ-40411813 (a PAM being developed by Janssen Pharmaceutica NV Beerse, Belgium, and Addex Therapeutics, Geneva, Switzerland) has been shown to have effects on negative symptoms in patients with schizophrenia in a phase II trial (85). Another mGluR2 receptor PAM (ADZ8529; AstraZeneca, Cambridge, UK) failed to separate from placebo in total, negative, and positive symptoms of schizophrenia in a phase II trial (86).

A recent paper published in *JAMA Psychiatry* in which researchers evaluated basimglurant (mGluR5 NAM) for patients with MDD with inadequate antidepressant responses in the current episode found that the primary endpoint (mean change in clinician-rated depression score from baseline to endpoint) was not met, but an antidepressant effect on patient-rated measures was found across secondary endpoints (87). However, an NAM targeting mGluR2/3 (decoglurant) was withdrawn, mainly owing to disappointing antidepressant efficacy (**Table 2**).

CONCLUSION

Although most techniques today indirectly measure glutamatergic neurotransmission *in vivo*, accumulating evidence has revealed that glutamatergic dysfunction plays a crucial role in major psychiatric disorders, such as schizophrenia, BD, and MDD (including TRD). Ketamine, esketamine, and many other pharmacological compounds targeting the glutamate system are available for human trials of major psychiatric disorders. However, longer-duration and larger studies are required to prove their clinical efficacy in different psychiatric diseases.

AUTHOR CONTRIBUTIONS

C-TL: conceived and designed the study; C-TL, K-CY, and W-CL: performed the analysis and review; C-TL, K-CY, and W-CL: wrote the paper; C-TL, K-CY, and W-CL: approved the paper.

FUNDING

This study was sponsored by grants from the Ministry of Science and Technology (MOST 106-2314-B-075-034-MY3).

The aforementioned organization had no influence on the study design, data collection, analysis, interpretation of results, writing of the report, or ultimate decision to submit the paper for publication.

ACKNOWLEDGMENTS

We would like to thank all of the research assistants, physicians, and personnel who helped us with this study.

REFERENCES

1. Lewerenz J, Maher P. Chronic glutamate toxicity in neurodegenerative diseases-what is the evidence? *Front Neurosci*. (2015) 9:469. doi: 10.3389/fnins.2015.00469
2. Gilad GM, Gilad VH, Wyatt RJ, Tizabi Y. Region-selective stress-induced increase of glutamate uptake and release in rat forebrain. *Brain Res*. (1990) 525:335–8. doi: 10.1016/0006-8993(90)90886-G
3. Moghaddam B. Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. *J Neurochem*. (1993) 60:1650–7. doi: 10.1111/j.1471-4159.1993.tb13387.x
4. Ohgi Y, Futamura T, Hashimoto K. Glutamate signaling in synaptogenesis and NMDA receptors as potential therapeutic targets for psychiatric disorders. *Curr Mol Med*. (2015) 15:206–21. doi: 10.2174/1566524015666150330143008
5. Li CT, Lu CF, Lin HC, Huang YZ, Juan CH, Su TP, et al. Cortical inhibitory and excitatory function in drug-naive generalized anxiety disorder. *Brain Stimul*. (2017) 10:604–8. doi: 10.1016/j.brs.2016.12.007
6. Reiner A, Levitz J. Glutamatergic signaling in the central nervous system: ionotropic and metabotropic receptors in concert. *Neuron* (2018) 98:1080–98. doi: 10.1016/j.neuron.2018.05.018
7. Furukawa H, Singh SK, Mancusso R, Gouaux E. Subunit arrangement and function in NMDA receptors. *Nature* (2005) 438:185–92. doi: 10.1038/nature04089
8. Davitz MS, Wu WE, Soher BJ, Babb JS, Kirov II, Huang J, et al. Quantifying global-brain metabolite level changes with whole-head proton MR spectroscopy at 3T. *Magn Reson Imaging* (2017) 35:15–9. doi: 10.1016/j.mri.2016.08.012
9. Harris AD, Saleh MG, Edden RA. Edited (1) H magnetic resonance spectroscopy *in vivo*: methods and metabolites. *Magn Reson Med*. (2017) 77:1377–89. doi: 10.1002/mrm.26619
10. Snyder J, Wilman A. Field strength dependence of PRESS timings for simultaneous detection of glutamate and glutamine from 1.5 to 7T. *J Magn Reson*. (2010) 203:66–72. doi: 10.1016/j.jmr.2009.12.002
11. Fu H, Chen Z, Josephson L, Li Z, Liang SH. Positron Emission Tomography (PET) ligand development for ionotropic glutamate receptors: challenges and opportunities for radiotracer targeting N-methyl-D-aspartate (NMDA), alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) and Kainate Receptors. *J Med Chem*. (2018) doi: 10.1021/acs.jmedchem.8b00714. [Epub ahead of print].
12. DeLorenzo C, DellaGioia N, Bloch M, Sanacora G, Nabulsi N, Abdallah C, et al. *In vivo* ketamine-induced changes in [11C]ABP688 binding to metabotropic glutamate receptor subtype 5. *Biological Psychiatry* (2015) 77:266–75. doi: 10.1016/j.biopsych.2014.06.024
13. Esterlis I, DellaGioia N, Pietrzak RH, Matuskey D, Nabulsi N, Abdallah CG, et al. Ketamine-induced reduction in mGluR5 availability is associated with an antidepressant response: an [(11)C]ABP688 and PET imaging study in depression. *Mol Psychiatry* (2018) 23:824–32. doi: 10.1038/mp.2017.58
14. Li CT, Chen MH, Lin WC, Hong CJ, Yang BH, Liu RS, et al. The effects of low-dose ketamine on the prefrontal cortex and amygdala in treatment-resistant depression: a randomized controlled study. *Hum Brain Map*. (2016) 37:1080–90. doi: 10.1002/hbm.23085
15. Mosconi L. Glucose metabolism in normal aging and Alzheimer's disease: methodological and physiological considerations for PET studies. *Clin Transl Imaging*. (2013) 1:217–33. doi: 10.1007/s40336-013-0026-y
16. Magistretti PJ, Pellerin L. Astrocytes couple synaptic activity to glucose utilization in the brain. *News Physiol Sci*. (1999) 14:177–82. doi: 10.1152/physiologyonline.1999.14.5.177
17. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. *J Physiol*. (1993) 471:501–19. doi: 10.1113/jphysiol.1993.sp019912
18. Valls-Solé J, Pascual-Leone A, Wassermann EM, Hallett M. Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalogr Clin Neurophysiol*. (1992) 85:355–64. doi: 10.1016/0168-5597(92)90048-G
19. Sanger TD, Garg RR, Chen R. Interactions between two different inhibitory systems in the human motor cortex. *J Physiol*. (2001) 530(Pt 2):307–17. doi: 10.1111/j.1469-7793.2001.0307l.x
20. Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H. Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. *J Physiol*. (1997) 498(Pt 3):817–23. doi: 10.1113/jphysiol.1997.sp021905
21. Liepert J, Schwenkreis P, Tegenthoff M, Malin JP. The glutamate antagonist riluzole suppresses intracortical facilitation. *J Neural Transm*. (1997) 104:1207–14. doi: 10.1007/BF01294721
22. Ziemann U, Tergau F, Wischer S, Hildebrandt J, Paulus W. Pharmacological control of facilitatory I-wave interaction in the human motor cortex. A paired transcranial magnetic stimulation study. *Electroencephalogr Clin Neurophysiol*. (1998) 109:321–30. doi: 10.1016/S0924-980X(98)00023-X
23. Cosgrove J, Newell TG. Recovery of neuropsychological functions during reduction in use of phencyclidine. *J Clin Psychol*. (1991) 47:159–69. doi: 10.1002/1097-4679(199101)47:1<159::AID-JCLP2270470125>3.0.CO;2-O
24. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* (1991) 148:1301–8. doi: 10.1176/ajp.148.10.1301
25. Testa CM, Standaert DG, Young AB, Penney JB. Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. *J Neurosci*. (1994) 14(5 Pt 2):3005–18. doi: 10.1523/JNEUROSCI.14-05-03005.1994
26. Cho HP, Garcia-Barrantes PM, Brogan JT, Hopkins CR, Niswender CM, Rodriguez AL, et al. Chemical modulation of mutant mGlu1 receptors derived from deleterious GRM1 mutations found in schizophrenics. *ACS Chem Biol*. (2014) 9:2334–46. doi: 10.1021/cb500560h
27. Meador-Woodruff JH, Healy DJ. Glutamate receptor expression in schizophrenic brain. *Brain Res Brain Res Rev*. (2000) 31:288–94. doi: 10.1016/S0165-0173(99)00044-2
28. Volk DW, Eggan SM, Lewis DA. Alterations in metabotropic glutamate receptor 1alpha and regulator of G protein signaling 4 in the prefrontal cortex in schizophrenia. *Am J Psychiatry* (2010) 167:1489–98. doi: 10.1176/appi.ajp.2010.10030318
29. Merritt K, Egerton A, Kempton MJ, Taylor MJ, McGuire PK. Nature of glutamate alterations in schizophrenia: a meta-analysis of proton magnetic resonance spectroscopy studies. *JAMA Psychiatry* (2016) 73:665–74. doi: 10.1001/jamapsychiatry.2016.0442
30. Salavati B, Rajji TK, Price R, Sun Y, Graff-Guerrero A, Daskalakis ZJ. Imaging-based neurochemistry in schizophrenia: a systematic review and implications for dysfunctional long-term potentiation. *Schizophr Bull*. (2015) 41:44–56. doi: 10.1093/schbul/sbu132
31. Iwata Y, Nakajima S, Plitman E, Mihashi Y, Caravaggio F, Chung JK, et al. Neurometabolite levels in antipsychotic-naive/free patients with schizophrenia: a systematic review and meta-analysis of (1)H-MRS studies. *Prog Neuropsychopharmacol Biol Psychiatry* (2018) 86:340–52. doi: 10.1016/j.pnpbp.2018.03.016
32. Egerton A, Broberg BV, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, et al. Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre (1)H-MRS study (OPTiMiSE). *Mol Psychiatry* (2018) 23:2145–55. doi: 10.1038/s41380-018-0082-9
33. Poels EM, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, et al. Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. *Mol Psychiatry* (2014) 19:20–9. doi: 10.1038/mp.2013.136
34. Lahti AC, Holcomb HH, Medoff DR, Tamminga CA. Ketamine activates psychosis and alters limbic blood flow in schizophrenia. *Neuroreport* (1995) 6:869–72. doi: 10.1097/00001756-199504190-00011

35. Holcomb HH, Lahti AC, Medoff DR, Weiler M, Tamminga CA. Sequential regional cerebral blood flow brain scans using PET with H2(15)O demonstrate ketamine actions in CNS dynamically. *Neuropsychopharmacology* (2001) 25:165–72. doi: 10.1016/S0893-133X(01)00229-9
36. Vollenweider FX, Leenders KL, Scharfetter C, Antonini A, Maguire P, Missimer J, et al. Metabolic hyperfrontality and psychopathology in the ketamine model of psychosis using positron emission tomography (PET) and [18F]fluorodeoxyglucose (FDG). *Eur Neuropsychopharmacol*. (1997) 7:9–24. doi: 10.1016/S0924-977X(96)00039-9
37. Narendran R, Frankle WG, Keefe R, Gil R, Martinez D, Slifstein M, et al. Altered prefrontal dopaminergic function in chronic recreational ketamine users. *Am J Psychiatry* (2005) 162:2352–9. doi: 10.1176/appi.ajp.162.12.2352
38. Stone JM, Erlandsson K, Arstad E, Bressan RA, Squassante L, Teneggi V, et al. Ketamine displaces the novel NMDA receptor SPET probe [(123)I]CNS-1261 in humans *in vivo*. *Nucl Med Biol*. (2006) 33:239–43. doi: 10.1016/j.nucmedbio.2005.12.001
39. Stone JM, Erlandsson K, Arstad E, Squassante L, Teneggi V, Bressan RA, et al. Relationship between ketamine-induced psychotic symptoms and NMDA receptor occupancy: a [(123)I]CNS-1261 SPET study. *Psychopharmacology* (2008) 197:401–8. doi: 10.1007/s00213-007-1047-x
40. Pilowsky LS, Bressan RA, Stone JM, Erlandsson K, Mulligan RS, Krystal JH, et al. First *in vivo* evidence of an NMDA receptor deficit in medication-free schizophrenic patients. *Mol Psychiatry* (2006) 11:118–9. doi: 10.1038/sj.mp.4001751
41. Radhu N, de Jesus DR, Ravindran LN, Zanjani A, Fitzgerald PB, Daskalakis ZJ. A meta-analysis of cortical inhibition and excitability using transcranial magnetic stimulation in psychiatric disorders. *Clin Neurophysiol*. (2013) 124:1309–20. doi: 10.1016/j.clinph.2013.01.014
42. Hasan A, Schneider M, Schneider-Axmann T, Ruge D, Retz W, Rösler M, et al. A similar but distinctive pattern of impaired cortical excitability in first-episode schizophrenia and ADHD. *Neuropsychobiology* (2013) 67:74–83. doi: 10.1159/000343912
43. Fitzgerald PB, Brown TL, Marston NA, Oxley TJ, de Castella A, Daskalakis ZJ, et al. A transcranial magnetic stimulation study of abnormal cortical inhibition in schizophrenia. *Psychiatry Res*. (2003) 118:197–207. doi: 10.1016/S0165-1781(03)00094-5
44. Beneyto M, Meador-Woodruff JH. Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. *Neuropsychopharmacology* (2008) 33:2175–86. doi: 10.1038/sj.npp.1301604
45. Beneyto M, Kristiansen LV, Oni-Orisan A, McCullumsmith RE, Meador-Woodruff JH. Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. *Neuropsychopharmacology* (2007) 32:1888–902. doi: 10.1038/sj.npp.1301312
46. Matosin N, Fernandez-Enright F, Frank E, Deng C, Wong J, Huang XF, et al. Metabotropic glutamate receptor mGluR2/3 and mGluR5 binding in the anterior cingulate cortex in psychotic and nonpsychotic depression, bipolar disorder and schizophrenia: implications for novel mGluR-based therapeutics. *J Psychiatry Neurosci*. (2014) 39:407–16. doi: 10.1503/jpn.130242
47. Nurnberger JI Jr, Koller DL, Jung J, Edenberg HJ, Foroud T, Guella I, et al. Identification of pathways for bipolar disorder: a meta-analysis. *JAMA Psychiatry* (2014) 71:657–64. doi: 10.1001/jamapsychiatry.2014.176
48. Blacker CJ, Lewis CP, Frye MA, Veldic M. Metabotropic glutamate receptors as emerging research targets in bipolar disorder. *Psychiatry Res*. (2017) 257:327–37. doi: 10.1016/j.psychres.2017.07.059
49. Gigante AD, Bond DJ, Lafer B, Lam RW, Young LT, Yatham LN. Brain glutamate levels measured by magnetic resonance spectroscopy in patients with bipolar disorder: a meta-analysis. *Bipolar Disord*. (2012) 14:478–87. doi: 10.1111/j.1399-5618.2012.01033.x
50. Chitty KM, Lagopoulos J, Lee RS, Hickie IB, Hermens DF. A systematic review and meta-analysis of proton magnetic resonance spectroscopy and mismatch negativity in bipolar disorder. *Eur Neuropsychopharmacol*. (2013) 23:1348–63. doi: 10.1016/j.euroneuro.2013.07.007
51. Szulc A, Wiedlocha M, Waszkiewicz N, Galinska-Skok B, Marcinowicz P, Gierus J, et al. Proton magnetic resonance spectroscopy changes after lithium treatment. Systematic review. *Psychiatry Res Neuroimaging* (2018) 273:1–8. doi: 10.1016/j.pscychresns.2018.01.001
52. Nugent AC, Diazgranados N, Carlson PJ, Ibrahim L, Luckenbaugh DA, Brutsche N, et al. Neural correlates of rapid antidepressant response to ketamine in bipolar disorder. *Bipolar Disord*. (2014) 16:119–28. doi: 10.1111/bdi.12118
53. Li CT, Hsieh JC, Wang SJ, Yang BH, Bai YM, Lin WC, et al. Differential relations between fronto-limbic metabolism and executive function in patients with remitted bipolar I and bipolar II disorder. *Bipolar Disord*. (2012) 14:831–42. doi: 10.1111/bdi.12017
54. Li CT, Tu PC, Hsieh JC, Lee HC, Bai YM, Tsai CF, et al. Functional dysconnection in the prefrontal-amygdala circuitry in unaffected siblings of patients with bipolar I disorder. *Bipolar Disord*. (2015) 17:626–35. doi: 10.1111/bdi.12321
55. Levinson AJ, Young LT, Fitzgerald PB, Daskalakis ZJ. Cortical inhibitory dysfunction in bipolar disorder: a study using transcranial magnetic stimulation. *J Clin Psychopharmacol*. (2007) 27:493–7. doi: 10.1097/jcp.0b013e31814ce524
56. McOmish CE, Pavey G, Gibbons A, Hopper S, Udawela M, Scarr E, et al. Lower [3H]LY341495 binding to mGlu2/3 receptors in the anterior cingulate of subjects with major depressive disorder but not bipolar disorder or schizophrenia. *J Affect Disord*. (2016) 190:241–8. doi: 10.1016/j.jad.2015.10.004
57. Feyissa AM, Chandran A, Stockmeier CA, Karolewicz B. Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2009) 33:70–5. doi: 10.1016/j.pnpbp.2008.10.005
58. Gray AL, Hyde TM, Deep-Soboslay A, Kleinman JE, Sodhi MS. Sex differences in glutamate receptor gene expression in major depression and suicide. *Mol Psychiatry* (2015) 20:1057–68. doi: 10.1038/mp.2015.91
59. Lee PH, Perlis RH, Jung JY, Byrne EM, Rueckert E, Siburian R, et al. Multi-locus genome-wide association analysis supports the role of glutamatergic synaptic transmission in the etiology of major depressive disorder. *Transl Psychiatry* (2012) 2:e184. doi: 10.1038/tp.2012.95
60. Lener MS, Niciu MJ, Ballard ED, Park M, Park LT, Nugent AC, et al. Glutamate and gamma-aminobutyric acid systems in the pathophysiology of major depression and antidepressant response to ketamine. *Biol Psychiatry* (2017) 81:886–97. doi: 10.1016/j.biopsych.2016.05.005
61. Arnone D, Mumuni AN, Jauhar S, Condon B, Cavanagh J. Indirect evidence of selective glial involvement in glutamate-based mechanisms of mood regulation in depression: meta-analysis of absolute prefrontal neuro-metabolic concentrations. *Eur Neuropsychopharmacol*. (2015) 25:1109–17. doi: 10.1016/j.euroneuro.2015.04.016
62. Luykx JJ, Laban KG, van den Heuvel MP, Boks MP, Mandl RC, Kahn RS, et al. Region and state specific glutamate downregulation in major depressive disorder: a meta-analysis of (1)H-MRS findings. *Neurosci Biobehav Rev*. (2012) 36:198–205. doi: 10.1016/j.neubiorev.2011.05.014
63. Li CT, Su TP, Wang SJ, Tu PC, Hsieh JC. Prefrontal glucose metabolism in medication-resistant major depression. *Br J Psychiatry* (2015) 206:316–23. doi: 10.1192/bjp.bp.113.140434
64. Li CT, Lin CP, Chou KH, Chen IY, Hsieh JC, Wu CL, et al. Structural and cognitive deficits in remitting and non-remitting recurrent depression: a voxel-based morphometric study. *Neuroimage* (2010) 50:347–56. doi: 10.1016/j.neuroimage.2009.11.021
65. Levinson AJ, Fitzgerald PB, Favalli G, Blumberger DM, Daigle M, Daskalakis ZJ. Evidence of cortical inhibitory deficits in major depressive disorder. *Biol Psychiatry* (2010) 67:458–64. doi: 10.1016/j.biopsych.2009.09.025
66. Mittal D, Chekuri L, Lu L, Fortney JC. Demographic, economic, and clinical correlates of depression treatment response in an underserved primary care population. *J Clin Psychiatry* (2014) 75:848–54. doi: 10.4088/JCP.13m08954
67. Croarkin PE, Nakonezny PA, Husain MM, Melton T, Buyukdura JS, Kennard BD, et al. Evidence for increased glutamatergic cortical facilitation in children and adolescents with major depressive disorder. *JAMA Psychiatry* (2013) 70:291–9. doi: 10.1001/2013.jamapsychiatry.24
68. Hackos DH, Lupardus PJ, Grand T, Chen Y, Wang TM, Reynen P, et al. Positive allosteric modulators of GluN2A-containing NMDARs with distinct modes of action and impacts on circuit function. *Neuron* (2016) 89:983–99. doi: 10.1016/j.neuron.2016.01.016
69. Barnes SA, Sheffler DJ, Semenova S, Cosford NDP, Bespalov A. Metabotropic glutamate receptor 5 as a target for the treatment of depression and smoking:

robust preclinical data but inconclusive clinical efficacy. *Biol Psychiatry* (2018) 83:955–62. doi: 10.1016/j.biopsych.2018.03.001
70. Molero P, Ramos-Quiroga JA, Martin-Santos R, Calvo-Sánchez E, Gutiérrez-Rojas L, Meana JJ. Antidepressant efficacy and tolerability of ketamine and esketamine: a critical review. *CNS Drugs* (2018) 32:411–20. doi: 10.1007/s40263-018-0519-3
71. Murrough JW, Wade E, Sayed S, Ahle G, Kiraly DD, Welch A, et al. Dextromethorphan/quinidine pharmacotherapy in patients with treatment resistant depression: a proof of concept clinical trial. *J Affect Disord*. (2017) 218:277–83. doi: 10.1016/j.jad.2017.04.072
72. Preskorn SH, Baker B, Kolluri S, Menniti FS, Krams M, Landen JW. An innovative design to establish proof of concept of the antidepressant effects of the NR2B subunit selective N-methyl-D-aspartate antagonist, CP-101,606, in patients with treatment-refractory major depressive disorder. *J Clin Psychopharmacol*. (2008) 28:631–7. doi: 10.1097/JCP.0b013e31818a6cea
73. Zarate CA Jr, Mathews D, Ibrahim L, Chaves JF, Marquardt C, Ukoh I, et al. A randomized trial of a low-trapping nonselective N-methyl-D-aspartate channel blocker in major depression. *Biol Psychiatry* (2013) 74:257–64. doi: 10.1016/j.biopsych.2012.10.019
74. Smith EG, Deligiannidis KM, Ulbricht CM, Landolin CS, Patel JK, Rothschild AJ. Antidepressant augmentation using the N-methyl-D-aspartate antagonist memantine: a randomized, double-blind, placebo-controlled trial. *J Clin Psychiatry* (2013) 74:966–73. doi: 10.4088/JCP.12m08252
75. Niciu MJ, Luckenbaugh DA, Ionescu DF, Richards EM, Vande Voort JL, Ballard ED, et al. Riluzole likely lacks antidepressant efficacy in ketamine non-responders. *J Psychiatr Res*. (2014) 58:197–9. doi: 10.1016/j.jpsychires.2014.07.022
76. Huang CC, Wei IH, Huang CL, Chen KT, Tsai MH, Tsai P, et al. Inhibition of glycine transporter-I as a novel mechanism for the treatment of depression. *Biol Psychiatry* (2013) 74:734–41. doi: 10.1016/j.biopsych.2013.02.020
77. Kantrowitz JT, Epstein ML, Lee M, Lehrfeld N, Nolan KA, Shope C, et al. Improvement in mismatch negativity generation during d-serine treatment in schizophrenia: Correlation with symptoms. *Schizophr Res*. (2018) 191:70–9. doi: 10.1016/j.schres.2017.02.027
78. Bugarski-Kirola D, Iwata N, Sameljak S, Reid C, Blaettler T, Millar L, et al. Efficacy and safety of adjunctive bitopertin versus placebo in patients with suboptimally controlled symptoms of schizophrenia treated with antipsychotics: results from three phase 3, randomised, double-blind, parallel-group, placebo-controlled, multicentre studies in the SearchLyte clinical trial programme. *Lancet Psychiatry* (2016) 3:1115–28. doi: 10.1016/S2215-0366(16)30344-3
79. Zarate CA Jr, Brutsche NE, Ibrahim L, Franco-Chaves J, Diazgranados N, Cravchik A, et al. Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. *Biol Psychiatry* (2012) 71:939–46. doi: 10.1016/j.biopsych.2011.12.010
80. Kantrowitz JT, Halberstam B, Gangwisch J. Single-dose ketamine followed by daily D-Cycloserine in treatment-resistant bipolar depression. *J Clin Psychiatry* (2015) 76:737–8. doi: 10.4088/JCP.14l09527
81. Park LT, Lener MS, Hopkins M, Iadorola N, Machado-Vieira R, Ballard E, et al. A double-blind, placebo-controlled, pilot study of riluzole monotherapy for acute bipolar depression. *J Clin Psychopharmacol*. (2017) 37:355–8. doi: 10.1097/JCP.0000000000000693
82. Goff DC, Lamberti JS, Leon AC, Green MF, Miller AL, Patel J, et al. A placebo-controlled add-on trial of the Ampakine, CX516, for cognitive deficits in schizophrenia. *Neuropsychopharmacology* (2008) 33:465–72. doi: 10.1038/sj.npp.1301444
83. Kinon BJ, Millen BA, Zhang L, McKinzie DL. Exploratory analysis for a targeted patient population responsive to the metabotropic glutamate 2/3 receptor agonist pomaglumetad methionil in schizophrenia. *Biol Psychiatry* (2015) 78:754–62. doi: 10.1016/j.biopsych.2015.03.016
84. Iwata Y, Nakajima S, Suzuki T, Keefe RS, Plitman E, Chung JK, et al. Effects of glutamate positive modulators on cognitive deficits in schizophrenia: a systematic review and meta-analysis of double-blind randomized controlled trials. *Mol Psychiatry* (2015) 20:1151–60. doi: 10.1038/mp.2015.68
85. Hopkins CR. Is there a path forward for mGlu(2) positive allosteric modulators for the treatment of schizophrenia? *ACS Chem Neurosci*. (2013) 4:211–3. doi: 10.1021/cn400023y
86. Litman RE, Smith MA, Doherty JJ, Cross A, Raines S, Gertsik L, et al. AZD8529, a positive allosteric modulator at the mGluR2 receptor, does not improve symptoms in schizophrenia: a proof of principle study. *Schizophr Res*. (2016) 172:152–7. doi: 10.1016/j.schres.2016.02.001
87. Quiroz JA, Tamburri P, Deptula D, Banken L, Beyer U, Rabbia M, et al. Efficacy and safety of basimglurant as adjunctive therapy for major depression: a randomized clinical trial. *JAMA Psychiatry* (2016) 73:675–84. doi: 10.1001/jamapsychiatry.2016.0838

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 06 February 2019
doi: 10.3389/fpsyt.2019.00025

D-Serine: Potential Therapeutic Agent and/or Biomarker in Schizophrenia and Depression?

Mary-Anne B. MacKay[1,2], *Maryana Kravtsenyuk*[1,2], *Rejish Thomas*[1], *Nicholas D. Mitchell*[1,2], *Serdar M. Dursun*[1,2] *and Glen B. Baker*[1,2]*

[1] Neurochemical Research Unit and Bebensee Schizophrenia Research Unit, Department of Psychiatry, University of Alberta, Edmonton, AB, Canada, [2] Neuroscience and Mental Health Institute, University of Alberta, Edmonton, AB, Canada

D-Serine is a potent co-agonist at the NMDA glutamate receptor and has been the object of many preclinical studies to ascertain the nature of its metabolism, its regional and cellular distribution in the brain, its physiological functions and its possible clinical relevance. The enzymes involved in its formation and catabolism are serine racemase (SR) and D-amino acid oxidase (DAAO), respectively, and manipulations of the activity of those enzymes have been useful in developing animal models of schizophrenia and in providing clues to the development of potential new antipsychotic strategies. Clinical studies have been conducted in schizophrenia patients to evaluate body fluid levels of D-serine and/or to use D-serine alone or in combination with antipsychotics to determine its effectiveness as a therapeutic agent. D-serine has also been used in combination with DAAO inhibitors in preclinical investigations, and interesting results have been obtained. Genetic studies and postmortem brain studies have also been conducted on D-serine and the enzymes involved in its metabolism. It is also of considerable interest that in recent years clinical and preclinical investigations have suggested that D-serine may also have antidepressant properties. Clinical studies have also shown that D-serine may be a biomarker for antidepressant response to ketamine. Relevant to both schizophrenia and depression, preclinical and clinical studies with D-serine indicate that it may be effective in reducing cognitive dysfunction.

Keywords: D-serine, D-amino acids, schizophrenia, depression, serine racemase, D-amino acid oxidase

OPEN ACCESS

Edited by:
Kenji Hashimoto,
Chiba University, Japan

Reviewed by:
Tianmei Si,
Peking University Sixth Hospital, China
Yilang Tang,
Emory University, United States

***Correspondence:**
Glen B. Baker
glen.baker@ualberta.ca

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *28 October 2018*
Accepted: *15 January 2019*
Published: *06 February 2019*

Citation:
MacKay M-AB, Kravtsenyuk M, Thomas R, Mitchell ND, Dursun SM and Baker GB (2019) D-Serine: Potential Therapeutic Agent and/or Biomarker in Schizophrenia and Depression? Front. Psychiatry 10:25. doi: 10.3389/fpsyt.2019.00025

INTRODUCTION

Several amino acids have a chiral center and thus can exist as D- and L-isomers. For many years, it was thought that only the L-isomers of these amino acids existed in mammalian tissue. However, it was discovered in the 1990s that relatively large quantities of free D-serine exist in the mammalian brain (1–3), although at lower concentrations than L-serine (1–5). Free D-aspartate and D-alanine (**Figure 1**) are also present in brain at levels much lower than those of D-serine and of their respective L-isomers, but still measureable (2, 3, 5, 7–13). Interestingly, it has been reported that all three of these D-amino acids may contribute to brain function (14–22) and may be useful as adjuncts in the therapy of schizophrenia (4, 7, 10, 17, 20–22).

The focus of this review is on D-serine and its possible involvement with both schizophrenia and depression. D-Serine is a potent coagonist at the N-methyl-D-aspartate (NMDA) glutamate receptor and appears to have a major modulatory role in NMDA receptor-mediated neurotransmission, neurotoxicity, synaptic plasticity, and cell migration (5, 8, 15, 18–23).

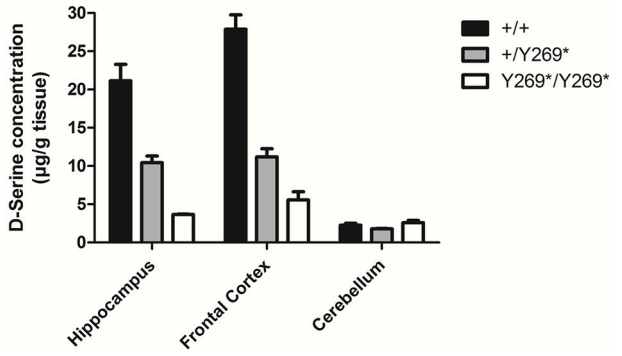

FIGURE 1 | Levels of D-serine in frontal cortex, hippocampus and cerebellum in mice with a nonsense mutation of exon 9 of the gene for SR: wild type, (+/+), heterozygous (+/Y269*), and mutant (Y269*/Y269*) mice. Behavioral deficits (impairment in prepulse inhibition, sociability, and spatial discrimination) in the mutant mice were worsened by an NMDA receptor antagonist and ameliorated by D-serine or clozapine [adapted from Labrie et al. (6)].

Considerable research now indicates that it may be a potential therapeutic agent and/or biomarker in both schizophrenia and major depressive disorder, as will be discussed in this review paper.

METHODS

Searches were done in PubMed and Web of Science covering the period 1990–2018 and the key phrases "D-serine and neuropsychiatric disorders," D-serine and schizophrenia," "D-serine and depression," "D-amino acids in neuropsychiatric disorders," and "D-serine and ketamine" were used in the searches. Only papers in English were used in the preparation of this review. The references obtained were screened by the authors to determine which would be best to put in this paper.

D-SERINE AS A POSSIBLE BIOMARKER AND/OR THERAPEUTIC AGENT IN SCHIZOPHRENIA

There is now a large body of evidence supporting hypofunction of NMDA glutamate receptors in schizophrenia (24–27). Because D-serine is such a potent coagonist at the NMDA receptor, there has been a great deal of interest in its role in the brain. D-Serine is present in glia (mainly astrocytes) and neurons. It has been proposed as both a glial transmitter (28, 29) and a neurotransmitter (30), and this has resulted in considerable controversy [see (29, 30) for an interesting discussion of the relevant importance of glia and neurons in the actions of D-serine]. Wolosker etal. (30) have proposed that astrocytes synthesize L-serine which then shuttles to neurons to be converted to D-serine.

The NMDA glutamate receptor requires not only glutamate but a coagonist in order to be activated. For many years, it was thought that glycine was the coagonist and the site at which it acts on the NMDA receptor is termed the glycine binding site. There is now considerable evidence, including a regional distribution more closely resembling that of NMDA receptors than is the case with glycine (4) and a stronger affinity than glycine for the glycine binding site on the NR1 subunit of the NMDA receptor (31, 32), indicating that D-serine may be more important in this regard. Because of the large body of evidence indicating hypofunctioning of the NMDA glutamate receptor in schizophrenia, D-serine has become of great interest to many researchers, and it has been proposed as the primary NMDA receptor coagonist in the forebrain and hippocampus (14). Glycine and D-serine appear to act at different NMDA receptor populations, D-serine at synaptic receptors and glycine at extrasynaptic receptors (33). It has been proposed that synaptic NMDA receptors are neuroprotective and that extrasynaptic receptors may promote cell death (34).

A number of preclinical studies in rodents have demonstrated that lowering brain levels of D-serine by reducing the activity of serine racemase (SR), the enzyme responsible for catalyzing formation of D-serine from L-serine (e.g., **Figure 1**), can produce symptoms reminiscent of clinical symptoms in schizophrenia: stereotypies, cognitive deficits, disruption of prepulse inhibition (measure of sensorimotor gating), persistent latent inhibition (measures inhibitory learning and cognitive flexibility), and deficits in social interaction (6, 19–22, 35). It has also been reported that SR knockout mice show a reduction of basal NMDA receptor activity and reduced arborization and spine density in dendrites (36). Chronic D-serine reverses expression of activity-related cytoskeleton-associated protein (Arc) and causes partial rescue of dendritic abnormalities in the same model (36).

Spatial and reversal memory deficits in Sprague-Dawley rats treated with the NMDA receptor antagonist phencyclidine (PCP) at different developmental stages can be reversed with D-serine administration (37). Hagiwara etal. (38) conducted an experiment with SR-inhibited mice where D-serine was given as a supplement in the preadolescent phase, and this had some benefit in preventing adult onset psychosis, suggesting the possibility of using D-serine supplementation in early intervention in humans. Fujita etal. (39) reported that juvenile and adolescent rodents that had been exposed prenatally to maternal immune activation showed reduced expression of hippocampal NMDA receptor subunits and onset of cognitive deficits as adults; supplementing their drinking water with D-serine from P28 to P56 reduced cognitive deficits.

For further information about SR and D-amino acid oxidase (DAAO), see the next section.

FORMATION AND CATABOLISM OF D-SERINE AND RELEVANCE OF THIS METABOLISM TO SCHIZOPHRENIA

D-Serine formation from L-serine is catalyzed via the enzyme SR and its catabolism is catalyzed by DAAO (40–46) (**Figure 2**).

In the brain, SR and D-serine are found in the same regions, i.e., high levels in forebrain areas such as cortex and hippocampus and very much lower levels in the cerebellum and brain stem (35). Activity of SR can be modulated through various α-amino-3-hydroxy-5-methylisoxazole-4-propionic

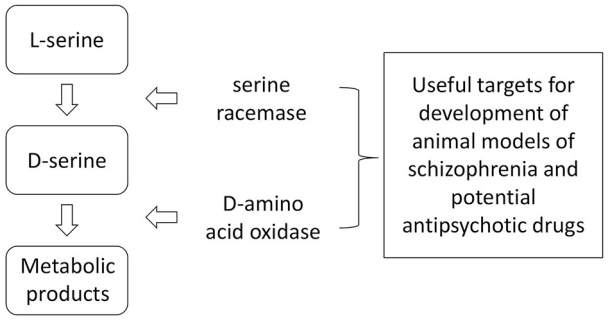

FIGURE 2 | Anabolism and catabolism of D-serine. Because of the relatively large number of factors interacting with these enzymes, there are numerous potential targets for influencing brain levels of D-serine through genetic or pharmacological manipulations.

acid (AMPA) receptor-dependent, metabotropic mGluR2/3/5 receptor-dependent mechanisms, divalent cations and the adenosine triphosphate (ATP) pathway, suggesting potential targets by which D-serine concentrations could be enhanced (47–49). AMPA-induced postsynaptic membrane depolarization builds up intracellular calcium concentrations and stimulates SR with the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) (48). Ma etal. (50) reported SR interactions with stargazin and the scaffolding protein PSD-95 and suggested that these proteins regulate NMDA receptor-AMPA receptor cross-talk in neurons. Lin etal. (51) proposed an association of D-serine with PSD-95 and NMDA receptors in postsynaptic neurons and with stability of glutamatergic synapses during development of cortical synapses. Glutamate-receptor-interacting-protein (GRIP) forms a complex with mGluR2/3 receptors, and receptor activation changes the conformation of SR, altering its function and decreasing production of D-serine (49).

Single nucleotide polymorphisms in the gene encoding protein interacting with C-kinase 1 (PICK1), a component of protein kinase C signaling, have been reported to be associated with a higher risk of schizophrenia, and it has been suggested that this link could be mediated through the interaction with SR (52). Ma etal. (53) proposed that pathogenic disruption of Disrupted-In-Schizophrenia-1 (DISC1)-SR binding can produce schizophrenia-like behavior by depleting D-serine levels. DISC1, the perturbation of which has been implicated in the pathophysiology of a number of mental disorders, including schizophrenia, major depressive disorder and bipolar disorder (54), binds to SR and stabilizes it. Using a mouse model of schizophrenia, Ma etal. (53) found that mutant DISC1 results in SR degradation and a D-serine deficiency. Svane etal. (55) administered D-serine to male and female rats and measured expression of nitric oxide synthase1 adaptor protein (NOS1AP) (overexpressed in cortex of patients with schizophrenia), D2 receptors and DISC1 and found that it affects expression of these three genes in a sex-specific manner. Mustafa etal. (56) reported that NO S-nitrosylates SR, mediating feedback inhibition of formation of D-serine. Interaction of SR with Colga 3, a member of the Colgin subfamily A, may also be important since Colga 3 decreases the ubquitylation of SR, resulting in protection of the SR from degradation by the ubiquitin-proteasomal system (57). In their SR knockout mouse model (58), Balu and Coyle (59) observed reduced binding of cyclic adenosine monophosphate (cAMP)-responsive element binding (CREB) to the promoter regions of genes for three molecules implicated in the pathophysiology of schizophrenia, namely brain-derived neurotrophic factor (BDNF), microRNA-132, and Arc (60). In a recent review article, Wolosker has provided a useful table of regulators of SR activity and D-serine production (61).

In contrast to the distribution of SR and D-serine in the brain, DAAO is most abundant in the cerebellum and brain stem and at much lower concentrations in pre-frontal cortex, hippocampus, and substantia nigra (13, 35). It has been reported that the activity of DAAO in postmortem brain tissue from humans with schizophrenia is increased over that of controls (62, 63). Labrie etal. (41) found that genetic loss of DAAO reverses the schizophrenia-like phenotypes in mice displaying these behaviors because of a mutation in the NR1 subunit of the NMDA receptor. DAAO has been proposed to be regulated by the protein product of gene G72 (64–67), and variations in pLG72 have been associated with schizophrenia (68–72). There has been some discussion in the literature about the various functions of pLG72 in the brain and the molecular details of its interaction with DAAO, and these matters have been reviewed comprehensively by Pollegiani etal. (73). Hashimoto etal. (74) found that administering a DAAO inhibitor and D-serine simultaneously reduces prepulse inhibition seen in mice given dizocilpine, a non-competitive NMDA receptor antagonist. Other researchers have also co-administered DAAO inhibitors in conjunction with D-serine in preclinical studies and suggested that DAAO inhibitors could be useful in schizophrenia by reducing the required dose for D-serine (40, 74–77).

CLINICAL STUDIES WITH D-SERINE IN SCHIZOPHRENIA

Levels of D-serine have been reported to be decreased in cerebrospinal fluid (CSF) and blood of schizophrenia patients (18, 78–84). Hashimoto etal. (84) reported that serum levels of D-serine in schizophrenia patients are lower than in controls, while D-serine serum levels in mood-stabilized bipolar disorder patients are higher than those in controls, and they felt that this situation may reduce misdiagnosis between these disorders. Further, plasma levels of D-serine and the D-/L-serine ratio were increased significantly by clozapine in schizophrenia responders (85).

Clinical studies have been conducted in schizophrenia with D-serine alone and as an adjunct to antipsychotics. In a 4-week open-label study it was reported that D-serine at high dose (60 mg/kg/day) resulted in improved neurocognitive function (86). The same group did follow-up studies on D-serine (87, 88). In one investigation they did a double-blind, placebo-controlled, parallel group randomized clinical trial on negative symptoms in at-risk individuals, and D-serine was given at a dose of 60 mg/kg/day in divided daily doses for 16-weeks; it was concluded

that D-serine should be useful for treatment of prodromal symptoms. In another double-blind study, they found that in schizophrenia patients treated with D-serine at 60 mg/kg/day, there was a significant improvement in mismatch negativity (MMN) (auditory mismatch), a neurophysiological biomarker for NMDA receptor activity. Ermilov etal. (89) compared high dose (3 g/day) D-serine with high dose olanzapine in treatment-resistant schizophrenia patients and concluded that a subgroup of patients could be maintained on D-serine. Interestingly, doses of D-serine as high as 4 g per day have been reported to cause no adverse effects (18).

Several clinical studies with D-serine have used it as a potential adjunctive drug (30–120 mg/kg/day) to antipsychotics. When added to non-clozapine antipsychotics in schizophrenia patients, D-serine has been reported to improve negative, positive, and cognitive symptoms (90–94) and to be relatively free of side effects even at high doses. A double-blind placebo-controlled study by Heresco-Levy etal. (91) examined the effect of supplemental D-serine in a group of patients stabilized on olanzapine or risperidone; at the end of 6-weeks of treatment, the D-serine intervention group demonstrated an improvement in cognitive, positive, and negative symptom domains and a significant alteration in depressive symptoms. Simultaneous measurement of serum amino acid concentrations did not detect any fluctuations of levels of any amino acids except D-serine. Meta-analyses of clinical trials comparing therapy with glycine modulatory site agonists, including D-serine, concluded that all of these NMDA receptor agonists significantly ameliorate symptoms in multiple domains, including cognitive and affective symptoms, when added to atypical antipsychotics except for clozapine (92–94). However, negative studies on D-serine adjunctive therapy in schizophrenia have also been reported in the literature (95–98). There have been inconsistent results as to the therapeutic benefit of D-serine used at 30 mg/kg/d to improve the negative and cognitive symptoms of the illness, with more consistent improvements found at doses of 60 mg/kg/d or higher. The higher doses of D-serine may be needed to adequately potentiate NMDA receptor-mediated activation of the receptor and to also achieve adequate serum levels and a subsequent predictive increase in brain concentrations.

It is interesting that D-serine addition to clozapine does not increase the efficacy of clozapine (99); this may be because clozapine releases D-serine and glutamate (100) and may have agonist or partial agonist activity at NMDA receptors (99–101). It is also possible that individuals on clozapine do not respond to D-serine since they are more often older and/or treatment-resistant (99).

One of the problems of treating symptoms of schizophrenia with D-serine is the fact that it is metabolized rapidly by DAAO, reducing its bioavailability and requiring administration of high doses, which could lead to peripheral neuropathies. There are also safety concerns that high concentrations of D-serine can cause potential nephrotoxicity related to its metabolism by DAAO as has been reported in rats that have developed acute tubular necrosis associated with higher doses of D-serine (102). However, in clinical studies that have administered D-serine (4-weeks duration) in doses up to 120 mg/kg/day, there have been no significant side effects (including nephrotoxicity) reported (86). In this particular study, one patient who received 120 mg/kg/d of D-serine did show 2+ proteinuria without glycosuria during the last week of treatment with no change in creatinine which completely resolved following the discontinuation of D-serine. In a 16-week intervention study by the same group using 60 mg/kg/d in clinically high-risk individuals, there were two patients who were discontinued in relation to abnormal renal values associated with treatment, with all other patients' renal abnormalities reported being resolved with continued treatment (87). The long-term side effects of D-serine beyond a 16-week treatment period are currently unknown. Additional studies that include longer time intervals should be conducted on this aspect of D-serine to ensure patient safety.

Studies on DAAO inhibitors indicate that they have limited effects on brain D-serine levels in mice, although there is some disagreement in the literature on this [see Guercio and Panizzutti (103) for a discussion]. However, as mentioned in the above section on enzymes involved in metabolism of D-serine, several researchers have now co-administered DAAO inhibitors in conjunction with D-serine in preclinical studies, and their findings suggest that DAAO inhibitors could be useful clinically in schizophrenia for reducing the required dose of D-serine and thus also reducing potential side effects associated with the administration of high D-serine (74–77). Sodium benzoate, a frequently used food preservative, is also a DAAO inhibitor and has been reported to have beneficial effects in schizophrenia when added to antipsychotics (104).

D-SERINE AND MAJOR DEPRESSIVE DISORDER

As indicated above, there is now a strong body of evidence indicating the possible involvement of D-serine in the etiology of schizophrenia and suggesting its potential as an antipsychotic or an adjunct to existing antipsychotics. It has thus been very interesting to see research results indicating that it may also have antidepressant effects and/or be a potential biomarker for depression and response to the antidepressant effects of ketamine. The possible mechanisms involved in the antidepressant actions of D-serine are the subject of another paper in this volume and of a paper by Chan etal. (105) and will not be discussed here in detail.

As mentioned above, in clinical studies investigating D-serine as an adjunct to antipsychotics, improvement in the affective symptoms of these patients was also often observed (e.g., 89–94). D-serine has now been reported to have antidepressant-like effects in rodent models of depression (105–107). Malkesman etal. (106) studied the behavioral effects in mice of a single, acute i.p. dose of D-serine in several tests of antidepressants, including the forced swim test, the female urine sniffing test following serotonin depletion and the learned helplessness paradigm and in mice lacking NR1 expression in excitatory neurons in the forebrain and found that D-serine gave a positive response in each of the behavioral tests but that the same behavioral tests

conducted in mice lacking NR1 expression did not respond to D-serine. Otte etal. (107) studied transgenic mice in which SR was overexpressed using paradigms of anxiety, depression, and cognition. These studies were done in the absence and presence of D-serine in the drinking water. D-Serine administration resulted in a reversal of the findings in these tests, suggesting a reduced proneness toward depression-related behavior. Wei etal. (108) studied the effects of D-serine and ketamine on rats in the forced swim test and proposed that D-serine produces antidepressant-like effects through the same mechanisms as ketamine. Wang etal. (109) found that chronic social defeat stress (CSDS) in mice induces expression of the D-serine transporter through epigenetic activation and decreases levels of D-serine in the hippocampus, leading to depression-like behavior.

The DAAO inhibitor sodium benzoate has been reported to have beneficial effects in a depressed patient (110) and acute administration of D-serine to healthy subjects has been reported to reduce subjective feelings of depression and anxiety as measured by Visual Analog Scales (111). Ishiwata etal. (112) reported that CSF levels of D-serine in depressed patients correlate negatively with severity of depression. In a comprehensive study on 70 depressed patients, Hashimoto etal. (113) found increased serum levels of both serine enantiomers compared to controls, while serum levels of glycine, glutamate, and glutamine did not differ between the depressed patients and controls. In contrast, Mitani etal. (114) had reported that plasma levels of D- and L-serine in major depressive disorder (MDD) patients were the same as those in healthy controls and levels of glutamate, glutamine, and glycine were higher in MDD patients than in controls. Hashimoto etal. (113) suggested that differences in severity of the depression between the two studies might account for some differences. Because of their findings of increased serum levels of D- and L-serine in MDD patients and a higher ratio of L-serine to glycine levels in the MDD patients, Hashimoto etal. (113) suggested abnormalities in synthesis and catabolism of serine enantiomers in MDD.

It is now well-accepted that intravenous administration of the NMDA receptor antagonist ketamine can produce a rapid improvement of symptoms of depression (115, 116). As noted above, several studies indicate that D-serine, an NMDA receptor coagonist, can also produce antidepressant effects. Malkesman etal. (106) state that the two drugs may activate common, convergent downstream targets, resulting in similar effects on protein expression and producing comparable changes in synaptic plasticity and dendritic remodeling. There are some interesting interactions between D-serine and ketamine reported. There is now evidence indicating that D-serine may be a predictive biomarker for antidepressant response to ketamine, with low plasma D-serine levels predicting a response to (R,S)-ketamine (117). Singh etal. (118) examined the effect of (R)- and (S)-ketamine on Alanine, Serine, Cysteine Transporter 2 (ASCT2)-mediated transport of D-serine in adrenal pheochromocytoma PC-12 and human neural astrocytoma 1321N1 cells, and primary neuronal cells in culture and reported that (S)-ketamine decreased cellular export by selectively inhibiting ASCT2; the authors suggested that this interaction might represent a source of dissociative effects seen with (R,S)-ketamine. Singh etal. (118) also incubated PC-12 cells with a variety of ketamine metabolites and determined the IC_{50} values associated with attenuation of intracellular D-serine and proposed to use the findings to help in the design of more efficient modulators of D-serine.

D-SERINE AND COGNITION

Serious cognitive deficits can occur in a number of psychiatric disorders, including both schizophrenia and MDD. It has been reported that depletion of D-serine levels in brain diminishes long-term potentiation (LTP), which is associated with learning and memory (19, 20). Genetic inactivation of SR in a mouse model and an acute stress model reduced brain levels of D-serine and produced cognitive deficits (6, 119). Intraperitoneal injection of D-serine results in improved social memory in rats (120) and improved recognition and working memory in mice (121). In a rodent model study following development of offspring after prenatal maternal infection [using poly(I:C)], it was reported that supplementing offspring at juvenile and adolescent stages with D-serine reduced cognitive deficits in adulthood (39). Panizzutti etal. (122) studied the association between serum D-serine levels and the results of intensive cognitive training in schizophrenia patients and found that in those patients receiving this training increased D-serine levels were positively correlated with improved global cognition and verbal learning, but such associations were not apparent with glycine. Despite the interesting findings mentioned above, D-serine is not used clinically as a cognitive enhancer; in a recent review, Guercio and Panizzutti (103) have discussed various factors, including pharmacokinetics and possible side effects that must be studied in more detail in order to increase the efficacy of D-serine.

SUMMARY

In contrast to the usual situation with D-amino acids, D-serine is abundant in the brain and appears to have important neuromodulatory roles. D-Serine is more potent than glycine as a coagonist at the NMDA receptor, has a regional distribution in the brain that is similar to that of NMDA receptors and appears to be more closely associated with synaptic NMDA receptors than glycine (which is more closely associated with non-synaptic NMDA receptors). There has been considerable controversy about the concentration and function of D-serine in glia vs. neurons, but evidence in recent years indicates that neurons are more relevant to D-serine action than originally thought. Synthesis and catabolism of D-serine are catalyzed by SR and DAAO, respectively. Regulation of these enzymes by various factors is quite complex. Decreased levels of D-serine in schizophrenia have been reported in several studies. Increasing D-serine levels in the brain may be an effective adjunct to antipsychotic treatment. D-Serine administered alone

or in combination with usual antipsychotics may be useful in treating schizophrenia, but the high doses required may cause peripheral neuropathies, although these have not been evident thus far using doses up to 4 g/day. Nonetheless, more studies at longer time intervals should be conducted on this aspect of D-serine to ensure patient safety. Several studies have been conducted with D-serine in combination with a DAAO inhibitor, with generally promising results obtained. Based on preclinical and clinical findings, D-serine also appears to have antidepressant properties. Several interesting pharmacodynamic and pharmacokinetic interactions between D-serine and ketamine have been reported, and, interestingly, evidence suggests that low plasma levels of D-serine may predict positive antidepressant response to ketamine. Several animal model and clinical studies also indicate that D-serine may be effective in reducing cognitive deficits, but that further study is necessary before considering it an effective cognitive enhancer for routine use in humans.

AUTHOR CONTRIBUTIONS

GB and M-AM wrote the first draft. All authors (GB, M-AM, MK, RT, NDM, SMD) contributed to the literature search and the editing of the manuscript.

FUNDING

Funding for GB's research has been provided by CIHR (MOP-86712) and the University of Alberta (FoMD TRIP research grant).

ACKNOWLEDGMENTS

The authors are grateful to the Canadian Institutes for Health Research, University of Alberta, and Alberta Health Services for research funding and to Trudy Valliere for assistance in typing this manuscript and Patricia Kent for preparing the figures.

REFERENCES

1. Hashimoto A, Nishikawa T, Hayashi T, Fujii N, Harada K, Oka T, etal. The presence of free D-serine in rat brain. *FEBS Lett.* (1992) 296:33–6. doi: 10.1016/0014-5793(92)80397-Y
2. Hashimoto A, Nishikawa T, Konno R, Niwa A, Yasumura Y, Oka T, etal. Free D-serine, D-aspartate, and D-alanine in central nervous system and serum in mutant mice lacking D-amino acid oxidase. *Neurosci Lett.* (1993) 152:33–6.
3. Hashimoto A, Oka T. Free D-aspartate and D-serine in the mammalian brain and periphery. *Prog Neurobiol.* (1997) 52:325–53. doi: 10.1016/S0301-0082(97)00019-1
4. Schell MJ, Brady RO Jr, Molliver ME, Snyder SH. D-Serine as a neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. *J Neurosci.* (1997) 17:1604–15. doi: 10.1523/JNEUROSCI.17-05-01604.1997
5. Fuchs SA, Berger R, Klomp LW, de Koning TJ. D-Amino acids in the central nervous system in health and disease. *Mol Genet Metab.* (2005) 85:168–80. doi: 10.1016/j.ymgme.2005.03.003
6. Labrie V, Fukumura R, Rastogi A, Fick LJ, Wang W, Boutros PC, etal. Serine racemase is associated with schizophrenia susceptibility in humans and in a mouse model. *Hum Mol Genet.* (2009) 18:3227–43. doi: 10.1093/hmg/ddp261
7. Kiriyama Y, Nochi H. D-Amino acids in the nervous and endocrine systems. *Scientifica* (2016) 2016:6494621. doi: 10.1155/2016/6494621
8. Billard JM. D-Amino acids in brain neurotransmission and synaptic plasticity. *Amino Acids* (2012) 43:1851–60. doi: 10.1007/s00726-012-1346-3
9. Mothet J-P, Snyder SH. Brain D-amino acids: a novel class of neuromodulators. *Amino Acids* (2012) 43:1809–10. doi: 10.1007/s00726-012-1403-y
10. Errico F, Mothet JP, Usiello A. D-Aspartate: an endogenous NMDA receptor agonist enriched in the developing brain with potential involvement in schizophrenia. *J Pharm Biomed Anal.* (2015) 116:7–17. doi: 10.1016/j.jpba.2015.03.024
11. Genchi G. An overview on D-amino acids. *Amino Acids* (2017) 49:1521–33. doi: 10.1007/s00726-017-2459-5
12. Li Z, Xing Y, Guo X, Cui Y. Development of an UPLC-MS/MS method for simultaneous quantitation of 11 D-amino acids in different regions of rat brain: application to a study on the associations of D-amino acid concentration changes and Alzheimer's disease. *J Chromatogr B Analyt Technol Biomed Life Sci.* (2017) 1058:40–6. doi: 10.1016/j.jchromb.2017.05.011
13. Verrall L, Burnet PWJ, Betts JF, Harrison PJ. The neurobiology of D-amino acid oxidase and its involvement in schizophrenia. *Mol Psychiatry* (2010) 15:122–37. doi: 10.1038/mp.2009.99
14. Labrie V, Roder JC. The involvement of the NMDA receptor D-serine/glycine site in the pathophysiology and treatment of schizophrenia. *Neurosci Behav Rev.* (2010) 34:351–72. doi: 10.1016/j.neubiorev.2009.08.002
15. Balu DT, Basu AC, Corradi JP, Cacace AM, Coyle JT. The NMDA receptor co-agonists, D-serine and glycine, regulate neuronal dendritic architecture in the somatosensory cortex. *Neurobiol Dis.* (2012) 45:671–82. doi: 10.1016/j.nbd.2011.10.006
16. Hashimoto K, Malchow B, Falkai P, Schmitt A. Glutamate modulators as potential therapeutic drugs in schizophrenia and affective disorders. *Eur Arch Psychiatry Clin Neurosci.* (2013) 263:367–77. doi: 10.1007/s00406-013-0399-y
17. Tsai GE, Yang P, Chang Y-C, Chong MY. D-Alanine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* (2006) 59:230–4. doi: 10.1016/j.biopsych.2005.06.032
18. Durrant AR, Heresco-Levy U. D-Serine in neuropsychiatric disorders: new advances. *Adv Psychiatry* (2014) 2014:859735. doi: 10.1155/2014/859735
19. Balu DT, Coyle JT. The NMDA receptor 'glycine modulatory site' in schizophrenia: D-serine, glycine, and beyond. *Curr Opin Pharmacol.* (2015) 20:109–15. doi: 10.1016/j.coph.2014.12.004
20. Bardaweel SK, Alzweiri M, Ishaqat AA. D-Serine in neurobiology: CNS neurotransmission and neuromodulation. *Can J Neurol Sci.* (2014) 41:164–76. doi: 10.1017/S031716710001653X
21. Wolosker H. NMDA receptor regulation by D-serine: new findings and perspectives. *Mol Neurobiol.* (2007) 36:152–64. doi: 10.1007/s12035-007-0038-6
22. Balu DT, Coyle JT. Neuronal D-serine regulates dendritic architecture in the somatosensory cortex. *Neurosci Lett.* (2012) 517:77–81. doi: 10.1016/j.neulet.2012.04.020
23. Kantrowitz JT, Epstein ML, Beggle O, Rohrig S, Lehrfeld JM, Revheim N, etal. Neurophysiological mechanisms of cortical plasticity impairments in schizophrenia and modulation by the NMDA receptor agonist D-serine. *Brain* (2016) 139:3281–95. doi: 10.1093/brain/aww262
24. Coyle JT, Tsai G, Goff D. Converging evidence of NMDA receptor hypofunction in the pathophysiology of schizophrenia. *Ann NY Acad Sci.* (2003) 1003:318–27. doi: 10.1196/annals.1300.020
25. Kantrowitz JT, Javitt DC. *N*-methyl-D-aspartate (NMDA) receptor dysfunction or dysregulation: the final common pathway on the road to schizophrenia? *Brain Res Bull.* (2010) 83:108–21. doi: 10.1016/j.brainresbull.2010.04.006
26. Lin CH, Lane HY, Tsai GE. Glutamate signaling in the pathophysiology and therapy of schizophrenia. *Pharmacol Biochem Behav.* (2012) 100:665–77. doi: 10.1016/j.pbb.2011.03.023
27. Balu DT. The NMDA receptor and schizophrenia: from pathophysiology to treatment. *Adv Pharmacol.* (2016) 76:351–82. doi: 10.1016/bs.apha.2016.01.006

28. Van Horn MR, Sild M, Ruthazer ES. D-Serine as a gliotransmitter and its role in brain development and disease. *Front Cell Neurosci.* (2013) 7:39. doi: 10.3389/fncel.2013.00039
29. Papouin T, Henneberger C, Rusakov DA, Oliet SHR. Astroglial versus neuronal D-serine: fact checking. *Trends Neurosci.* (2017) 40:517–20. doi: 10.1016/j.tins.2017.05.007
30. Wolosker H, Balu DT, Coyle JT. The rise and fall of the D-serine-mediated gliotransmission hypothesis. *Trends Neurosci.* (2016) 39:712–21. doi: 10.1016/j.tins.2016.09.007
31. Matsui T, Sekiguchi M, Hashimoto A, Tomita U, Nishikawa T, Wada K. Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. *J Neurochem.* (1995) 65:454–8. doi: 10.1046/j.1471-4159.1995.65010454.x
32. Shleper M, Kartvelishvily E, Wolosker H. D-Serine is the dominant endogenous coagonist for NMDA receptor neurotoxicity in organotypic hippocampal slices. *J Neurosci.* (2005) 25:9413–7. doi: 10.1523/JNEUROSCI.3190-05.2005
33. Papouin T, Ladépêche L, Ruel J, Sacchi S, Labasque M, Hanini M, etal. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. *Cell* (2012) 150:633–46. doi: 10.1016/j.cell.2012.06.029
34. Hardingham GE, Hading H. Synaptic versus extrasynaptic NMDA receptor signaling: implications for neurodegenerative disorders. *Nat Rev Neurosci.* (2010) 11:682–96. doi: 10.1038/nrn2911
35. Labrie V, Wong AHC, Roder J. Contributions of the D-serine pathway to schizophrenia. *Neuropharmacology* (2012) 62:1484–503. doi: 10.1016/j.neuropharm.2011.01.030
36. Balu DT, Coyle JT. Chronic D-serine reverses arc expression and partially rescues dendritic abnormalities in a mouse model of NMDA receptor hypofunction. *Neurochem Lett.* (2014) 75:76–8. doi: 10.1016/j.neuint.2014.05.015
37. Anderson JD, Poulzet B. Spatial memory deficits induced by perinatal treatment of rats with PCP and reversal effect of D-serine. *Neuropsychopharmacology* (2004) 29:1080–90. doi: 10.1038/sj.npp.1300394
38. Hagiwara H, Iyo M, Hashimoto K. Neonatal disruption of serine racemase causes schizophrenia-like behavioral abnormalities in adulthood: clinical rescue by D-serine. *PLoS ONE* (2013) 8:e62438.28. doi: 10.1371/journal.pone.0062438
39. Fujita Y, Ishima T, Hashimoto K. Supplementation with D-serine prevents the onset of cognitive deficits in adult offspring after maternal immune activation. *Sci Rep.* (2016) 6:37261. doi: 10.1038/srep37261
40. Sershen H, Hashim A, Dunlop DS, Suckow RF, Cooper TB, Javitt DC. Modulating NMDA receptor function with D-amino acid oxidase inhibitors: understanding functional activity in PCP-treated mouse model. *Neurochem Res.* (2016) 41:398–408. doi: 10.1007/s11064-016-1838-8
41. Labrie V, Wang W, LabrieBarger SW, Baker GB, Roder JC. Genetic loss of D-amino acid oxidase activity reverses schizophrenia-like phenotypes in mice. *Genes Brain Behav.* (2010) 9:11–25. doi: 10.1111/j.1601-183X.2009.00529.x
42. Wolosker H, Blackshaw S, Snyder SH. Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-*N*-methyl-D-aspartate neurotransmission. *Proc Natl Acad Sci USA.* (1999) 96:13409–14. doi: 10.1073/pnas.96.23.13409
43. Caldinelli L, Molla G, Bracci L, Lelli B, Pileri S, Cappelletti P, etal. Effect of ligand binding on human D-amino acid oxidase: implications for the development of new drugs for schizophrenia treatment. *Protein Sci.* (2010) 19:1500–12. doi: 10.1002/pro.429
44. Snell K, Fell DA. Metabolic control analysis of mammalian serine metabolism. *Adv Enzyme Regul.* (1990) 30:13–32. doi: 10.1016/0065-2571(90)90006-N
45. Coyle JT, Balu DT. The role of serine racemase in the pathophysiology of brain disorders. *Adv Pharmacol.* (2018) 82:35–56. doi: 10.1016/bs.apha.2017.10.002
46. Verrall L, Walker M, Rawlings N, Benzel I, Kew JN, Harrison PJ, etal. D-Amino acid oxidase and serine racemase in human brain: normal distribution and altered expression in schizophrenia. *Eur J Neurosci.* (2007) 26:1657–69. doi: 10.1111/j.1460-9568.2007.05769.x
47. Kim PM, Aizawa H, Kim PS, Huang AS, Wickramasinghe SR, Kashani AH, etal. Serine racemase: activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration. *Proc Nat Acad Sci USA.* (2005) 6:2105–10. doi: 10.1073/pnas.0409723102
48. Mothet JP, Pollegioni L, Ouanounou G, Martineau M, Fissier P, Baux G. Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. *Proc Natl Acad Sci USA.* (2005) 102:5606–11. doi: 10.1073/pnas.0408483102
49. Baumgart F, Manchen JM, Rodrigez-Crezpo I. Insights into the activation of brain serine racemase by the multi-PDZ domain glutamate receptor inter-acting protein, divalent cations and ATP. *FEBS J.* (2007) 274:4561–71. doi: 10.1111/j.1742-4658.2007.05986.x
50. Ma TM, Paul BC, Fu C, Hu S, Zhu H, Blackshaw S, etal. Serine racemase regulated by binding to stargazin and PSD-95: potential N-methyl-D-aspartate-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (NMDA-AMPA) glutamate neurotransmission cross-talk. *J Biol Chem.* (2014) 289:29631–41. doi: 10.1074/jbc.M114.571604
51. Lin H, Jocobi AA, Anderson SA, Lynch DR. D-Serine and serine racemase are associated with PSD-95 and glutamatergic synapse stability. *Front Cell Neurosci.* (2016) 10:34. doi: 10.3389/fncel.2016.00034
52. Fujii K, Maeda K, Hikida T, Mustafa AK, Balkissoon R, Xia J, etal. Serine racemase binds to PICK1: Potential relevance to schizophrenia. *Mol Psychiatry* (2006) 11:150–7. doi: 10.1038/sj.mp.4001776
53. Ma TM, Abazyan S, Abazyan B, Nomura J, Yang C, Seshadri S, etal. Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. *Mol Psychiatry* (2013) 18:557–67. doi: 10.1038/mp.2012.97
54. Lipina TV, Roder JC. Disrupted-in-Schizophrenia-1 (DISC1) interactome and mental disorders: impact of mouse models. *Neurosci Biobehav Rev.* (2014) 45:271–94. doi: 10.1016/j.neubiorev.2014.07.001
55. Svane KC, Asis EK, Omelchenko A, Kunnath AH, Brzustowicz LM, Silverstein SM, etal. D-Serine administration affects nitric oxide synthase 1 adaptor protein and DISC1 expression in sex-specific manner. *Mol Cell Neurosci.* (2018) 89:20–32. doi: 10.1016/j.mcn.2018.03.011
56. Mustafa AK, Kumar M, Selvakumar B, Ho GP, Ehmsen JT, Barrow RK, etal. Nitric oxide S-nitrosylates serine 56racemase, mediating feedback inhibition of D-serine formation. *Proc Natl Acad Sci USA.* (2007) 104:2950–5. doi: 10.1073/pnas.0611620104
57. Dumin E, Bendikov I, Foltyn VN, Misumi Y, Ikehara Y, Kartvelishvily E, etal. Modulation of D-serine levels via ubiquitin-dependent proteasomal degradation of serine racemase. *J Biol Chem.* (2006) 281:20291–302. doi: 10.1074/jbc.M601971200
58. Balu DT, Li Y, Puhl MD, Benneyworth MA, Basu AC, Takagi S, etal. Multiple risk pathways for schizophrenia converge in serine racemase knockout mice, a mouse model of NMDA receptor hypofunction. *Proc Natl Acad Sci USA.* (2013) 110:E2400–9. doi: 10.1073/pnas.1304308110
59. Balu DT, Coyle JT. Altered CREB binding to activity-dependent genes in serine racemase deficient mice, a mouse model of schizophrenia. *ACS Chem Neurosci.* (2018) 9:2205–9. doi: 10.1021/acschemneuro.7b00404
60. DeVito LM, Balu DT, Kanter BR, Lykken C, Basu AC, Coyle JT, etal. Serine racemase deletion disupts memory for order and alters cortical dendritic morpholgy. *Genes Brain Behav.* (2011) 10:210–22. doi: 10.1111/j.1601-183X.2010.00656.x
61. Wolosker H. The neurobiology of D-serine signaling. *Adv Pharmacol.* (2018) 82:325–48. doi: 10.1016/bs.apha.2017.08.010
62. Madiera C, Freitas ME, Vargas-Lopes C, Wolosker H, Panizzutti R. Increased D-amino acid oxidase (DAAO) activity in schizophrenia. *Schizophr Res.* (2008) 101:76–83. doi: 10.1016/j.schres.2008.02.002
63. Habl G, Zink M, Petrolanu G, Bauer M, Schneider-Azmann T, von Wilmsdorff M, etal. Increased D-amino acid oxidase expression in the bilateral hippocampal CA4 of schizophrenic patients: a postmortem study. *J Neural Transm.* (2009) 116:1657–65. doi: 10.1007/s00702-009-0312-z
64. Muller DJ, Zai CC, Shinkai T, Strauss J, Kennedy JL. Association between the DAOA/G72 gene and bipolar disorder and meta-analysis in bipolar disorder and schizophrenia. *Bipolar Disord.* (2011) 13:198–207. doi: 10.1111/j.1399-5618.2011.00905.x
65. Prata DP, Papagni SA, Mechelli A, Fu CH, Kambeitz J, Picchioni M, etal. Effect of D-amino acid oxidase activator (DAOA; G72) on brain

function during verbal fluency. *Hum Brain Mapp.* (2012) 33:143–53. doi: 10.1002/hbm.21198
66. Sacchi S, Cappelletti P, Giovannardi S, Pollegionni L. Evidence for the interaction of D-amino acid oxidase with pLG72 in a glial cell line. *Mol Cell Neurosci.* (2011) 48:20–8. doi: 10.1016/j.mcn.2011.06.001
67. Birolo L, Sacchi S, Smaldone G, Molla G, Leo G, Caldinelli L, etal. Regulating levels of the neuromodulator D-serine in human brain: structural insight into pLG72 and D-amino acid oxidase interaction. *FEBS J.* (2016) 283:3353–70. doi: 10.1111/febs.13809
68. Detera-Wadleight SD, McMahon FJ. G72/G30 in schizophrenia and bipolar disorder: a review and meta-analysis. *Biol Psychiatry* (2006) 60:106–14. doi: 10.1016/j.biopsych.2006.01.019
69. Sacchi S, Bernasooni M, Martineau M, Mothet JP, Ruzzene M, Pilone MS, etal. pLG72 modulates intracellular D-serine levels through its interaction with D-amino acid oxidase: effect on schizophrenia susceptibility. *J Biol Chem.* (2008) 283:22244–56. doi: 10.1074/jbc.M709153200
70. Cappelletti P, Campomenosi P, Pollegioni L, Sacchi S. The degradation (by distinct pathways) of human D-amino acid oxidase and its interacting partner pLG72-two key proteins in D-serine catabolism in the brain. *FEBS J.* (2014) 281:708–23. doi: 10.1111/febs.12616
71. Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, etal. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. *Proc Natl Acad Sci USA.* (2002) 99:13675–80. doi: 10.1073/pnas.182412499
72. Lin CH, Chang HT, Chen YJ, Lin CH, Huang CH, Tun R, etal. Distinctively higher plasma G72 protein levels in patients with schizophrenia than in the healthy individuals. *Mol Psychiatry* (2014) 19:636–7. doi: 10.1038/mp.2013.80
73. Pollegioni L, Plubelli L, Molla G, Rosini E. D-Amino acid oxidase-pLG72 interaction and D-serine modulation. *Front Mol Biosci.* (2018) 5:3. doi: 10.3389/fmolb.2018.00003
74. Hashimoto K, Fujita Y, Horio M, Kunitachi S, Iyo M, Ferraris D, etal. Co-administration of a D-amino acid oxidase inhibitor potentiates the efficacy of D-serine in attenuating prepulse inhibition deficits after administration of dizocilpine. *Biol Psychiatry* (2009) 65:1103–6. doi: 10.1016/j.biopsych.2009.01.002
75. Ferraris D, Duvall B, Ko Y-S, Thomas AG, Rojas C, Majer P, etal. Synthesis and biological evaluation of D-amino acid oxidase inhibitors. *J Med Chem.* (2008) 51:3357–9. doi: 10.1021/jm800200u
76. Ferraris DV, Tsukamoto T. Recent advances in the discovery of D-amino acid oxidase inhibitors and their therapeutic utility in schizophrenia. *Curr Pharm Des.* (2011) 17:103–11. doi: 10.2174/138161211795049633
77. Sacchi S, Rosini E, Pollegioni L, Molla G. D-Amino acid oxidase inhibitors as a novel class of drugs for schizophrenia therapy. *Curr Pharm Des.* (2013) 19:2499–511. doi: 10.2174/1381612811319140002
78. Hashimoto K, Fukushima T, Shimizu E, Kamotsu N, Watanabe H, Shinoda N, etal. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. *Arch Gen Psychiatry* (2003) 60:572–6. doi: 10.1001/archpsyc.60.6.572
79. Hashimoto K, Engberg G. Shimizu E, Nordin C, Lindstrom LH, Iyo M. Reduced D-serine to total serine ratio in the cerebrospoinal fluid of drug naïve schizophrenic patients. *Prog Neuropsychopharmacol Biol Psychiatry* (2005)29:767–9. doi: 10.1016/j.pnpbp.2005.04.023
80. Bendikov I, Nadri C, Amar S, Panizzutti R, De Miranda J, Wolosker H, etal. A CSF and postmortem brain study of D-serine metabolic parameters in schizophrenia. *Schizophr Res.* (2007) 90:41–51. doi: 10.1016/j.schres.2006.10.010
81. Ohnuma T, Sakai Y, Maeshima H, Hatano T, Hanzawa R, Abe S. Changes in plasma glycine, L-serine and D-serine levels in patients with schizophrenia as their clinical symptoms improve: results from the Juntendo University Schizophrenia Projects (JUSP). *Prog Neuropsychopharmacol Biol Psychiatry* (2008) 32:1905–12. doi: 10.1016/j.pnpbp.2008.07.022
82. Calcia, MA, Madeira C, Alheira FV, Silva TC, Tannos FM, Vargas-Lopes C, etal. Plasma levels of D-serine in Brazilian individuals with schizophrenia. *Schizophr Res.* (2012) 142:83–7. doi: 10.1016/j.schres.2012.09.014
83. Cho SE, Na K-S, Cho S-J, Kang SG. Low D-serine levels in schizophrenia: a systematic review and meta-analysis. *Neurosci Lett.* (2016) 634:42–51. doi: 10.1016/j.neulet.2016.10.006
84. Hashimoto K. Serine enantiomers as diagnostic biomarkers in schizophrenia and bipolar disorder. *Eur Arch Psychiatry Clin Neurosci.* (2016) 266:83–5. doi: 10.1007/s00406-015-0602-4
85. Yamamori H, Hashimoto R, Fujita Y, Numata S, Yasuda Y, Fujimoto M, etal. Changes in plasma D-serine, L-serine, and glycine levels in treatment-resistant schizophrenia before and after clozapine treatment. *Neurosci Lett.* (2014) 582:93–8. doi: 10.1016/j.neulet.2014.08.052
86. Kantrowitz JT, Malhotra AK, Cornblatt B, Silipo G, Ball A, Suckow RF. High dose D-serine in the treatment of schizophrenia. *Schizophr Res.* (2010) 121:125–30. doi: 10.1016/j.schres.2010.05.012
87. Kantrowitz JT, Woods SW, Petkova E, Cornblatt B, Corcoran CM, Chen H, etal. D-Serine for the treatment of negative symptoms in individuals at clinical high risk of schizophrenia: a pilot, double-blind, placebo-controlled, randomized parallel group mechanistic proof-of-concept trial. *Lancet Psychiatry* (2015) 2:403–12. doi: 10.1016/S2215-0366(15)00098-X
88. Kantrowitz JT, Epstein ML, Lee M, Lehrfeld N, Nolan KA, Shope C, etal. Improvement in mismatch negativity generation during D-serine treatment in schizophrenia: correlation with symptoms. *Schizophr Res.* (2018) 191:70–9. doi: 10.1016/j.schres.2017.02.027
89. Ermilov M, Gelfin E, Levin R, Lichtenberg P, Hashimoto K, Havitt DC, etal. A pilot double-blind comparison of D-serine and high-dose olanzapine in treatment-resistant patients with schizophrenia. *Schizophr Res.* (2013) 150:604–5. doi: 10.1016/j.schres.2013.09.018
90. Tsai G, Yang P, Chung LC, Lange N, Coyle JT. D-serine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* (1998) 44:1081–9.
91. Heresco-Levy U, Javitt DC, Ebstein R, Vass A, Lichtenberg P, Bar G, etal. D-Serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. *Biol Psychiatry* (2005) 57:577–85. doi: 10.1016/j.biopsych.2004.12.037
92. Tsai, GE, Lin PY. Strategies to enhance N-methyl-D-aspartate receptor –mediated neurotransmission in schizophrenia, a critical review and meta-analysis. *Curr Pharm Design* (2010) 16:522–37. doi: 10.2174/138161210790361452
93. Choi KH, Wykes T, Kurtz MM. Adjunctive pharmacotherapy for cognitive deficits in schizophrenia: meta-analytical investigation of efficacy. *Br J Psychiatry* (2013) 203:172–8. doi: 10.1192/bjp.bp.111.107359
94. Singh SP, Singh V. Meta-analysis of the efficacy of adjunctive NMDA receptor modulators in chronic schizophrenia. *CNS Drugs* (2011) 25:859–85. doi: 10.2165/11586650-000000000-00000
95. Lane H-Y, Chang Y-C, Liu Y-C, Chiu C-C, Tsai GE. Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. *Arch Gen Psychiatry* (2005) 62:1196–204. doi: 10.1001/archpsyc.62.11.1196
96. Weiser M, Heresco-Levy U, Davidson M, Javitt DC, Werbeloff N, Gershon AA, etal. A multicenter, add-on randomized controlled trial of low-dose D-serine for negative and cognitive symptoms of schizophrenia. *J Clin Psychiatry* (2012) 73:e728–34. doi: 10.4088/JCP.11m07031
97. Nunes EA, MacKenzie EM, Rossolatos D, Perez-Parada J, Baker GB, Dursun SM. D-Serine and schizophrenia: an update. *Expert Rev Neurother.* (2012) 12:801–12. doi: 10.1586/ern.12.65
98. Iwata Y, Nakajima S, Suzuki T, Keefe RS, Plitman E, Chung JK, etal. Effects of glutamate positive modulators on cognitive deficits in schizophrenia: a systematic review and meta-analysis of double-blind randomized controlled trials. *Mol Psychiatry* (2015) 20:1151–60. doi: 10.1038/mp.2015.68
99. Tsai GE, Yang P, Chung LC, Tsai IC, Tsai CW, Coyle JT. D-serine added to clozapine for the treatment of schizophrenia. *Am J Psychiatry* (1999) 156:1822–5.
100. Tanahashi S, Yamamura S, Nakagawa M, Motomura E, Okada M. Clozapine, but not haloperidol, enhances glial D-serine and L-glutamate release in rat frontal cortex and primary cultured astrocytes. *Br J Pharmacol.* (2012) 165:1543–55. doi: 10.1111/j.1476-5381.2011.01638.x
101. Chen L, Yang CR. Interaction of dopamine D1 and NMDA receptors mediates acute clozapine potentiation of glutamate EPSPs in rat prefrontal cortex. *J Neurophysiol.* (2002) 87:2324–36. doi: 10.1152/jn.2002.87.5.2324

102. Carone FA, Ganote CE. D-serine nephrotoxicity. The nature of proteinuria, glucosuria, and aminoaciduria in acute tubular necrosis. *Arch Pathol.* (1975) 12:658–62.
103. Guercio GD, Panizzutti R. Potential and challenges for the clinical use of D-serine as a cognitive enhancer. *Front Psychiatry* (2018) 9:14. doi: 10.3389/fpsyt.2018.00014
104. Lane HY, Lin CH, Green MF, Hellemann G, Huang CC, Chen PW, etal. Add-on treatment of benzoate for schizophrenia: a randomized, double-blind, placebo-controlled trial of a D-amino acid oxidase inhibitor. *JAMA Psychiatry* (2013) 70:1267–75. doi: 10.1001/jamapsychiatry.2013.2159
105. Chan SY, Matthews E, Burnet PWJ. On or off?: modulating the N-methyl-D-aspartate receptor in major depression. *Front Mol Neurosci.* (2017) 9:169. doi: 10.3389/fnmol.2016.00169
106. Malkesman O, Austin DR, Tragon T, Wang G, Rompala G, Hamidi AB, etal. Acute D-serine treatment produces antidepressant-like effects in rodents. *Int J Neuropsychopharmacol.* (2012) 15:1135–48. doi: 10.1017/S1461145711001386
107. Otte DM, Barcena de Arellano ML, Bilkei-Gorzo A, Albayram O, Imbeault S, Jeung H, etal. Effects of chronic D-serine elevation on animal models of depression and anxiety-related behavior. *PLoS ONE* (2013) 8:e67131. doi: 10.1371/journal.pone.0067131
108. Wei IH, Chen KT, Tsai MH, Wu CH, Lane HY, Huang CC. Acute amino acid D-serine administration, similar to ketamine, produces antidepressant-like effects through identical mechanisms. *J Agric Food Chem.* (2017) 65:10792–803. doi: 10.1021/acs.jafc.7b04217
109. Wang J, Zhang K, Chen X, Liu X, Teng H, Zhao M, etal. Epigenetic activation of *ASCT2* in the hippocampus contributes to depression-like behavior by regulating D-serine in mice. *Front Mol Neurosci.* (2017) 10:139. doi: 10.3389/fnmol.2017.00139
110. Lai C-H, Lane H-Y, Tsai GE. Clinical and cerebral volumetric effects of sodium benzoate, a D-amino acid oxidase inhibitor, in a drug-nave patient with major depression. *Biol Psychiatry* (2012) 71:e9–10. doi: 10.1016/j.biopsych.2011.10.034
111. Levin R, Dor-Abarbanel AE, Edelman S, Durrant AR, Hashimoto K, Javitt DC, etal. Behavioral and cognitive effects of the N-methyl-D-aspartate receptor co-agonist D-serine and healthy humans: initial findings. *J Psychiatr Res.* (2015) 61:188–95. doi: 10.1016/j.jpsychires.2014.12.007
112. Ishiwata S, Hattori K, Sasayama D, Teraishi T, Miyakawa T, Yokota Y, etal. Cerebrospinal fluid D-serine concentrations in major depressive disorder negatively correlate with depression severity. *J Affect Disord.* (2018) 226:155–62. doi: 10.1016/j.jad.2017.09.035
113. Hashimoto K, Yoshida T, Ishikawa M, Fujita Y, Niitsu T, Nakazato M, etal. Increased serum levels of serine enantiomers in patients with depression. *Acta Neuropsychiatr.* (2016) 28:173–8. doi: 10.1017/neu.2015.59
114. Mitani H, Shirayama Y, Yamada T, Maeda K, Ashby CR Jr, Kawahara R. Correlation between plasma levels of glutamate, alanine and serine with severity of depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2006) 30:1155–8. doi: 10.1016/j.pnpbp.2006.03.036
115. Zarate CA Jr, Niciu MJ. Ketamine for depression: evidence, challenges and promise. *World Psychiatry* (2015) 14:348–50. doi: 10.1002/wps.20269
116. Krystal JH, Sanacora G, Duman RS. Rapid-acting glutamatergic antidepressants: the path to ketamine and beyond. *Biol Psychiatry* (2013) 73:1133–41. doi: 10.1016/j.biopsych.2013.03.026
117. Moaddel R, Luckenbaugh DA, Xie Y, Villaseñor A, Brutsche NE, Machado-Vieira R, etal. D-serine plasma concentration is a potential biomarker of (R,S)-ketamine antidepressant response in subjects with treatment-resistant depression. *Psychopharmacology* (2015) 232:399–409. doi: 10.1007/s00213-014-3669-0
118. Singh NS, Bernier M, Camandola S, Khadeer MA, Moaddel R, Mattson MP, etal. Enantioselective inhibition of D-serine transport by (S)-ketamine. *Br J Pharmacol.* (2015) 172:4546–59. doi: 10.1111/bph.13239
119. Guercio GD, Bevictori I, Vargas-Lopes C, Madeira C, Oliveira A, Carvalho VF, etal. D-serine prevents cognitive deficits induced by acute stress. *Neuropharmacology* (2014) 861:1–8. doi: 10.1016/j.neuropharm.2014.06.021
120. Shimazaki T, Kaku A, Chaki S. D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats. *Psychopharmacology* (2010) 209:263–70. doi: 10.1007/s00213-010-1794-y
121. Bado P, Madeira C, Vargas-Lopes C, Moulin TC, Wastlewska-Sampaio AP, Maretti L, etal. Effects of low-dose D-serine on recognition and working memory in mice. *Psychopharmacology* (2011) 218:461–70. doi: 10.1007/s00213-011-2330-4
122. Panizzutti R, Fisher M, Garrett C, Man WH, Sena W, Madeira C, etal. Association between increased serum D-serine and cognitive gains induced by intensive cognitive training in schizophrenia. *Schizophr Res.* (2018) doi: 10.1016/j.schres.2018.04.011. [Epub ahead of print].

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 12 February 2019
doi: 10.3389/fpsyt.2019.00014

The Effects of Acupuncture on Glutamatergic Neurotransmission in Depression, Anxiety, Schizophrenia, and Alzheimer's Disease: A Review of the Literature

Cheng-Hao Tu[1], *Iona MacDonald*[1] *and Yi-Hung Chen*[1,2,3]*

[1] *Graduate Institute of Acupuncture Science, China Medical University, Taichung, Taiwan,* [2] *Department of Photonics and Communication Engineering, Asia University, Taichung, Taiwan,* [3] *Chinese Medicine Research Center, China Medical University, Taichung, Taiwan*

Neuropsychiatric disorders, including depression, anxiety, schizophrenia, and Alzheimer's disease (AD), are diseases that are directly or indirectly associated with cerebral dysfunction and contribute significantly to disability in adult populations worldwide. Important limitations surround the currently available pharmacologic agents for neuropsychiatric disorders and, moreover, many patients fail to respond to these therapies. Acupuncture might be a complementary therapy for neuropsychiatry disorders. In this review, we investigate the current evidence for the treatment efficacy of acupuncture in depression, anxiety, schizophrenia, and AD. Secondly, we review recent advances in understanding of the dysregulated glutamate system underlying the pathophysiology of these disorders. Finally, we discuss the ways in which acupuncture treatment can potentially modulate glutamate receptors and excitatory amino acid transporters. We conclude that the treatment effects of acupuncture may be underpinned by its intervention in the dysregulated glutamate system. Further preclinical and clinical studies are needed to clarify the possible mechanisms of acupuncture in these neuropsychiatric disorders and to establish protocols for treatment guidelines.

Keywords: acupuncture, glutamate, neuropsychiatric disorders, Alzheimer's disease, depression, anxiety, schizophrenia

OPEN ACCESS

Edited by:
Kenji Hashimoto,
Chiba University, Japan

Reviewed by:
Marek Schwendt,
University of Florida, United States
Li-Hong Kong,
Hubei University of Chinese Medicine, China
Tuya Bao,
Beijing University of Chinese Medicine, China

***Correspondence:**
Yi-Hung Chen
yihungchen@mail.cmu.edu.tw

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *01 September 2018*
Accepted: *10 January 2019*
Published: *12 February 2019*

Citation:
Tu C-H, MacDonald I and Chen Y-H (2019) The Effects of Acupuncture on Glutamatergic Neurotransmission in Depression, Anxiety, Schizophrenia, and Alzheimer's Disease: A Review of the Literature. Front. Psychiatry 10:14. doi: 10.3389/fpsyt.2019.00014

INTRODUCTION

Neuropsychiatry focuses on illness relating to altered cognition, mood, or behavior caused by cerebral dysfunction with neuronal pathological changes (e.g., dysregulated neurotransmitter systems or tissue damage), or abnormal physiological conditions (e.g., hyper/hypoglycemia or hypoxia). These changes in health conditions have profound effects upon individuals and society (1). Neuropsychiatric disorders contribute to over 10% of disability worldwide, exceeding the morbidity rates associated with cardiovascular disease or cancer (2). In developed-market economies, 25% of all disability has been attributed to neuropsychiatric disorders (2). In 2016, ∼18% of adults aged ≥18 years in the United States of America had any mental illness in the past year and ∼4% had a serious mental illness in that period (3, 4). A serious mental illness is defined as a diagnosable mental, behavioral, or emotional disorder (e.g., major depressive disorder

[MDD], schizophrenia) causing serious functional impairment and substantially interfering with or limiting one or more major life activities, such as maintaining interpersonal relationships, activities of daily living, self-care, employment, and recreation (3, 4). Similarly, a systematic review of epidemiological data from 16 European countries estimated in 2005 that 27% of the adult population (18–65 years of age) had experienced at least one mental disorder (e.g., substance use, psychosis, depression, anxiety, or eating disorder) in the past 12 months (5). Despite the high burden of psychiatric illness, only a subset of these people receive the mental health services that they need. For instance, according to data from the World Health Organization (WHO) European Region, 3 out of 4 people with MDD are inadequately treated (6). The majority of psychiatric disorders are mild or moderate, but if left untreated, the evidence suggests that they can develop into more serious illness (2).

Important limitations surround the currently available treatments for neuropsychiatric disorders. For instance, first-line pharmacotherapy for MDD typically consists of a selective serotonin reuptake inhibitor, a serotonin and norepinephrine reuptake inhibitor, or the norepinephrine-dopamine reuptake inhibitor bupropion, alone or in combination with psychotherapy. These therapies are associated with low remission rates and high dropout rates (7). As for the treatment of schizophrenia, the typical and atypical antipsychotics are mostly effective for the positive symptoms (hallucinations, delusions); cognitive and negative symptoms (deficits in working memory and attention, negative affect, and anhedonia) are largely unresponsive to current pharmacologic therapies. Moreover, serious side effects limit the use of some otherwise effective medications (8).

ACUPUNCTURE IN NEUROPSYCHIATRY TREATMENT

Acupuncture has long been used in Chinese medicine to treat numerous neuropsychiatric conditions, from acute delirium to post-stroke spasticity. The two forms of acupuncture manipulation that are used clinically are manual acupuncture (MA) and electroacupuncture (EA) (9). Traditional acupuncturists commonly use MA, whereby they insert the acupuncture needle into the acupoint to a certain depth and rotate it by hand. In EA, the needles are connected to an electrical stimulator that delivers a stimulating current to the acupoints. Another method that is also described as EA is the positioning of a surface electrode on the skin at the acupoint, without insertion of acupuncture needles. For this review, the evidence on the use of acupuncture in neuropsychiatry treatment is limited to investigations using MA and EA, with acupuncture needle insertion.

Numerous clinical reports from various sources, including the non-Western scientific literature, attest to the efficacy of acupuncture in depression (10–13), anxiety disorders (14, 15), schizophrenia (16–19), and Alzheimer's disease (AD) (20–23). Although much of this evidence is widely acknowledged to be of varying quality, many reports attest to the efficacy and safety of acupuncture treatment (24–27). In experienced hands, acupuncture is a safe therapy with a low risk of adverse events. Serious and potentially life-threatening acupuncture-related complications, including transmission of infections, pneumothorax, cardiovascular lesions, and hemorrhage or hematomas in the central nervous system (CNS), are very rarely reported (28). In a large study from Germany that included 2.2 million acupuncture sessions in 229,230 patients, the overall incidence of acupuncture-related adverse events was 8.6%, among which 2.2% of the patients required medical treatment (29). The vast majority of adverse events were due to minor bleeding/hematomas (6.1%), pain (1.7%), or vegetative symptoms such as vertigo, or nausea (0.7%). A review of three Chinese trials involving nearly 2,000 treatments identified instances of subcutaneous hematoma, bleeding and needle site pain, and reported that elderly people seem to be at greater risk of such adverse events (30).

Acupuncture for the Treatment of Depression

The effectiveness of acupuncture in depression has been extensively investigated with various sets of acupuncture points and treatment parameters (e.g., duration, frequency, and number of treatment sessions). A recent Cochrane systematic review that included 64 studies (7,104 participants) examined the effectiveness of acupuncture for the treatment of depression (10). The evidence indicated that acupuncture treatment may moderately or slightly lower the severity of depression compared with treatment as usual and control acupuncture (invasive, non-invasive sham controls), respectively, although the quality of the evidence was judged as being mostly low or very low. Other reviews have also described evidence in support of MA and EA as generally beneficial, safe and well-tolerated as monotherapy in MDD and post-stroke depression, but the evidence is insufficient to support the use of acupuncture in combination with antidepressants (31, 32). Two Chinese studies examined the effectiveness of acupuncture and moxibustion (a treatment involving burning of the dried Chinese herb mugwort or *Artemesia vulgaris* to apply heat onto or very close to an acupoint) in relieving psychological distress in 163 patients with depression and sought to determine whether gender-related differences exist in response to acupuncture and moxibustion (12, 13). The authors reported that acupuncture and moxibustion can significantly improve distress at even as late as 3 months after the completion of treatment, and that the level of efficacy is higher among females than males. Hence, acupuncture treatment in depression may improve depressive symptoms of depression and endure for some months.

Moreover, sleep status may improve in patients with depression after acupuncture treatment. A recent meta-analysis that included 18 randomized clinical trials (RCTs) involved 1,678 adults given acupuncture for depression-related insomnia and found significant improvements in sleep quality with acupuncture compared with Western medicine (11). When acupuncture was given as an adjunctive therapy with Western medicine, both depression and insomnia were improved (11). However, in another RCT involving 150 patients with

residual insomnia associated with MDD, traditional acupuncture needling produced only mild treatment effects that were similar to those of minimal acupuncture and placebo acupuncture (33). There were no significant group-by-time interactions during the 5-week post-treatment period. Thus, the psychological effect of acupuncture might play an important role in the treatment of acupuncture in depression-related insomnia.

Acupuncture for the Treatment of Anxiety

The effectiveness of acupuncture in anxiety has been widely investigated, using various sets of acupuncture points and treatment parameters. A narrative review published by the British Acupuncture Council reported that regular acupuncture and EA treatments improved anxiety symptoms (34). However, significant differences between the protocols used in regular acupuncture and EA made it hard to rule out a general beneficial or possible placebo effect. A more recent systematic literature review that included 32 English-language clinical and preclinical studies published between 2000 and 2010 also reported significant, positive results with acupuncture treatment for anxiety (14). Although the quality of these studies was variable, the authors suggested that patients who are resistant to conventional interventions (e.g., cognitive behavioral therapy) may prefer acupuncture treatment. Thus, acupuncture treatment may have positive effects on the symptoms of anxiety.

Acupuncture may also be of benefit in anxiety-related insomnia. A Canadian study reported that acupuncture significantly improved sleep quality in patients with anxiety and insomnia (15). At the end of acupuncture treatment, urine 6-sulfatoxymelatonin (a metabolite of melatonin) levels were normalized and several polysomnographic measures, as well as self-reported fatigue, sleepiness, anxiety, and level of depression, were significantly improved. Combined with the evidence of treatment efficacy in depression-related insomnia, acupuncture may have broader utility in neuropsychiatric disorders with impaired quality of sleep.

Interestingly, acupuncture treatment may also reduce anxiety levels in medical conditions other than neuropsychiatry. An early study reported that auricular acupuncture reduced state anxiety in patients waiting for surgery by a significantly greater extent than either body or sham acupuncture (35). Another study has reported that both body and auricular acupuncture effectively reduce preoperative anxiety; self-rated anxiety scores were significantly reduced from baseline in both groups (36). Possible underlying mechanisms remain unclear. One plausible explanation is that acupuncture regulates the autonomic nervous system. For instance, acupuncture appears to modulate heart rate variability, a non-invasive indicator of changes in autonomic state. In patients with mild depression or anxiety, verum acupuncture but not sham acupuncture was associated with significant reductions from baseline in mean resting heart rate at 5 and 15 min after needle application, with a trend toward an increase in high frequency (HF; 0.15–0.4 Hz) and a decrease in low frequency (LF; 0.04–0.15 Hz) spectral power (37). These results suggest that verum acupuncture modulates autonomic activity in response to alterations of internal and external environments, and thus reduces overall anxiety in patients with depression or anxiety.

However, it should be recognized that the interpretation of results from clinical trials investigating acupuncture interventions in depression and anxiety disorders is complicated by different interventions, different comparators used against acupuncture interventions, and the small sample sizes in many trials (38). These shortcomings prevent any accurate assessment of acupuncture for these conditions or a true comparison of the relative effectiveness of different treatment regimens. Moreover, the data are difficult to interpret from those studies where needling at specific and non-specific points have yielded similar outcomes (38).

The evidence consulted for depression and anxiety is summarized in **Table 1**.

Acupuncture for the Treatment of Schizophrenia

Compared with depression and anxiety, relatively few studies have addressed the efficacy of acupuncture in schizophrenia. A meta-analysis of 13 RCTs including 954 patients, all from China, provides positive evidence for the effectiveness of acupuncture (with or without EA or moxibustion) in treating the symptoms of schizophrenia (19). Some of the RCTs reported that acupuncture plus drug therapy significantly improved auditory hallucinations, positive symptoms and response rates compared with antipsychotics alone or in combination with sham EA. In a recent systematic review of data from 26 studies (1,181 participants) reporting limited evidence for the use of adjunctive acupuncture therapy in the treatment of positive, negative and cognitive symptoms, the authors point out the importance of differences between quantitative and qualitative changes (16). The limited evidence for treatment efficacy may be partly due to the fact that positive symptoms still exist, but patients are suffering less. Other evidence suggests that individualized acupuncture is beneficial for patients with schizophrenia as an adjunctive treatment with routine care (18). After completing individualized acupuncture sessions, patients reported improvements in symptoms of schizophrenia, side effects of medication, energy, motivation, sleep, addictions and other associated physical problems. A case study has reported that positive and negative symptoms can be improved for up to 3 months after add-on acupuncture treatment (17). A more recent case study describes how add-on acupuncture treatment improved general psychopathology and negative symptoms but not positive symptoms (39). Thus, individualized add-on acupuncture treatment may help to alleviate symptoms of schizophrenia.

Interestingly, the review by van den Noort et al. also describes beneficial effects with add-on acupuncture in accompanying sleep disorders (16). Several of the studies in that review reported improvements in subjective and objective sleep measurements. Acupuncture treatment appears to have similar effects to but is safer than zopiclone, a prescription medication for sleep disorder. Thus, acupuncture treatment may improve sleep dysregulation in schizophrenia.

TABLE 1 | Overview of characteristics of included studies evaluating acupuncture for depression and anxiety.

Smith et al. (10)	
Participants	7,104 adult men and women with depression.
Interventions	This Cochrane Review included 64 RCTs comparing acupuncture vs. control acupuncture (invasive, non-invasive sham controls), no treatment/wait-list, medication, psychological therapy, or standard care. Modes of treatment included acupuncture, EA and laser acupuncture.
Outcomes	Acupuncture may moderately reduce the severity of depression when compared with treatment as usual/no treatment. Use of acupuncture may reduce the severity of depression in comparison with control acupuncture. The very low quality of evidence limits the interpretation of the effects of acupuncture vs. medication and psychological therapy. Risks of adverse events with acupuncture are also unclear, as most trials did not report adverse events.
	Review paper
Fan et al. (13)	
Participants	163 patients with depression.
Interventions	Participants were randomized either to acupuncture plus moxibustion using a method that soothes the liver and regulates the mind (Group A; $n = 54$), to an acupoint shallow puncturing group with a shorter duration of moxibustion (Group B; $n = 56$), or to a non-acupoint shallow puncturing group (Group C; $n = 53$). All participants received twice-weekly treatment for 12 weeks. Group A received conventional acupuncture at the 4 gate points [Hegu [LI4]; Taichong [LR3]; Baihui [GV20]; Yintang [GV29]], with moxibustion applied with a moxa cone directly to the 4 flower points (Geshu [BL17]; Danshu [BL19]). Group A also received intradermal needling at Xinshu (BL15) and Ganshu (BL18). For Group B, the same acupoints were used as those for Group A, but with a shallower needling depth and a shorter duration of moxibustion. Group C received acupuncture at acupoints 10 mm lateral to the acupoints used in Group A, with the same manipulation method as in Group B.
Outcomes	At 1 and 3 months after treatment, several Symptom Checklist 90 (SCL-90) scores were improved from baseline by a significantly greater extent in Group A compared with Group C (all $p < 0.05$). SCL-90 scores for depression, anxiety and hostility were improved by a significantly greater extent at 1 and 3 months in Group A compared with Group B (all $p < 0.05$).
Fan et al. (12)	
Participants	163 patients with depression.
Interventions	Acupuncture and moxibustion soothing liver and regulating mind treatment (Group A); acupoint shallow puncturing (Group B); and non-acupoint shallow puncturing (Group C). Acupoints used in Group A were bilateral Hegu (LI4), bilateral Taichong (LR3), Baihui (GV20), and Yingtang (GV29). Needles were inserted vertically to a depth of 10–12 mm for LI4 and LR3; the needles were inserted at an angle of 30° to a depth of 4–5 mm for GV20 and GV29. After recording the *de qi* needling sensation (e.g., numbness, soreness, distention, heaviness during acupuncture stimulation), acupuncture used the *dao qi* technique at the acupoints, involving lifting, thrusting, and rotation of the needle. Needles remained in place for 30 min. Moxibustion involved the four flower points (bilateral BL17 and BL19). A round moxa cone (1 cm diameter, 1 cm high) was placed and ignited on the selected points, which were smeared evenly with Wanhua oil. Five cones were applied to each point. Bilateral BL15 Xinshu and BL18 Ganshu were selected for intradermal needling. An intradermal needle was completely inserted at each point and retained for 2 days. All treatments were given twice weekly for 12 weeks, with intervals lasting more than 48 h between two treatments. For Group B, the same acupoints were used as those in Group A, with a 2- to 3-mm needling depth. No lifting, thrusting, or rotating was performed, and the needle sensation was not required. Moxibustion was done for a shorter duration and only 1–2 mm of the intradermal needle body was inserted into the points. Group C received acupuncture at points 10 mm lateral to LI4 and LR3 and 10 mm to the left side of GV20 and GV29 during the acupuncture procedure, 10 mm lateral to BL17 and BL19 during the moxibustion procedure, and 10 mm lateral to BL15 and BL18 during the intradermal needling procedure, with the same manipulation method as that in Group B.
Outcomes	At 1 and 3 months after treatment, SCL-90 and HAMD scores differed by sex between Group A and Group B; women were more sensitive to the efficacy of the soothing liver and regulating mind treatment compared with other methods.
Dong et al. (11)	
Participants	1,678 adults (aged 18–75 years) with depression-related insomnia.
Interventions	11 RCTs compared acupuncture with Western medicine; 5 RCTs compared medication alone or in combination with acupuncture; 2 RCTs compared acupuncture with sham or placebo acupuncture control.
Outcomes	A pooled analysis of 10 RCTs that reported PSQI scores demonstrated significant improvements with acupuncture over Western medicine. When acupuncture was combined with Western medicine, sleep quality, and depression were both improved by a greater extent compared with Western medicine alone. Improvements from baseline in HAMD scores did not differ significantly between acupuncture treatment and Western medicine.
	Review paper
Chung et al. (33)	
Participants	150 outpatients with residual insomnia associated with MDD.
Interventions	Nine × 30-min sessions of acupuncture, given 3 times a week for 3 consecutive weeks. Participants were asked to continue the same type and dosage of antidepressants throughout the study period. They were randomized to "Traditional acupuncture" based on TCM principles of acupuncture treatment for insomnia, minimal acupuncture, or placebo acupuncture. The traditional acupuncture group was needled at bilateral Ear Shenmen, Sishencong (EX-HN1), Anmian (EX), Neiguan (PC6), Shenmen (HT7), Sanyinjiao (SP6), and unilateral Yintang (EX-HN3) and Baihui (GV20), using the TCM style of acupuncture. Acupoints on the head, hands and legs were treated with 0.25 × 25-mm needles; ear acupoints on ears were treated with 0.20 × 25-mm needles; insertion depths varied between 2 and 25 mm, depending on the points selected. *De qi* was achieved if possible. All needles were connected to an electric stimulator that delivered a constant current (0.4 ms, square-wave, brief-pulse stimulus of 4-Hz frequency). The needles were left for 30 min and then removed.

(Continued)

TABLE 1 | Continued

	The minimal acupuncture group was needled at points that have no therapeutic effects according to TCM theory and superficially to avoid *de qi*. Points on the limbs included bilateral "forearm," 1 inch lateral to the middle point between Shaohai (HE3) and Shenmen (HE7); "upper arm," 1 inch lateral to Tianfu (LU 3); and "lower leg," 0.5 inch dorsal to Xuanzhong (GB39). Points on the head included bilateral "head," the middle point between Shuaigu (GB8) and Touwei (ST8); "forehead", the middle point between Touwei (ST8) and Yangbai (GB14); "neck", the middle point between Tianyou (TB16) and Tianrong (SI17); and "ear," a point on the helix, inferior to the apex. Other treatment conditions and electrostimulation were the same as in the acupuncture group. The placebo acupuncture group received Streitberger placebo needles placed at sites 1 inch beside the acupuncture points used in the acupuncture group, with the aim of avoiding an acupressure effect. The needles were connected to an electric stimulator but with zero frequency and amplitude.
Outcomes	Traditional acupuncture needling produced only mild hypnotic effects that were similar to those of minimal acupuncture and placebo acupuncture. A high proportion of patients in each treatment group remained clinically significantly affected by insomnia after treatment.
British Acupuncture Council (34)	
Participants	Patients with anxiety disorders or depression.
Interventions	Verum (regular) acupuncture compared with sham acupuncture and EA.
Outcomes	Sham-controlled studies indicated that anxiety improved with both regular acupuncture and EA treatments. Significant differences between the protocols used in regular acupuncture and EA precluded any general beneficial or possible placebo effect. Moreover, although the findings from most controlled studies indicated a general anxiety-reducing effect of acupuncture, these were regarded by the reviewers as inconclusive because of study design problems, including the absence of standardized symptom rating scales in most studies, limited follow-up, and poorly defined differences between protocols used in different studies.
	Review book
Errington-Evans (14)	
Participants	Healthy volunteers, patients with anxiety disorders, and animal models of anxiety, from 32 English-language articles published between 2000 and 2010.
Interventions	TCM and non-TCM acupoints were used in patients, with a lack of detail provided by the studies as to point selection and treatment methodology. Animal studies assessed treatment outcomes in rodents subjected to chronic mild stress (controls) vs. no stress (a "natural" group), acupuncture vs. sham acupuncture.
Outcomes	The poor quality of the methodology reporting prevents any treatment recommendation.
	Review paper
Spence et al. (15)	
Participants	18 adult volunteers reporting having symptoms of insomnia for $\geq$2 continuous years immediately prior to the study and with scores >50 (anxiety range) on the Zung Anxiety Self Rating Scale. The study participants did not satisfy DSM-IV criteria for any particular anxiety disorder.
Interventions	Acupuncture therapy was given for 5 weeks (2 sessions/week, 10 sessions in total). Each acupuncture session lasted ~1 h. Two consecutive overnight polysomnographic studies were performed at baseline (before treatment) and at the end of the acupuncture treatment. Mood and cognitive efficiency was tested by the Toronto Alexithymia Scale, the Stanford Sleepiness Scale (SSS), and a 7-item Fatigue Scale. Anxiety was assessed by the State-Trait Anxiety Inventory and depressive symptoms by the Center for Epidemiological Studies Depression Scale (CES-D). On the following morning, immediately after waking, each subject completed a standard post-sleep questionnaire, the SSS, and the Fatigue Scale. Approximately 20 min after awakening, subjects assessed their level of fatigue and sleepiness on the Fatigue Severity Scale, the Epworth Sleepiness Scale, the Toronto Western Hospital Fatigue Questionnaire, the Fatigue Scale, and the FaST Adjective Checklist. They were also tested for accuracy and time to complete a complex verbal reasoning task. During both test phases, urine samples were tested for changes in endogenous levels of melatonin over 24 h.
Outcomes	At 5 weeks, acupuncture treatment was associated with significant reductions in state and trait anxiety scores, significant improvements in polysomnographic measures of sleep onset latency, arousal index, total sleep time, and sleep efficiency. Nocturnal endogenous melatonin secretion was significantly increased.
Acupoints	No mention of acupoints.
Wang and Kain (35)	
Participants	55 patients with preoperative anxiety.
Interventions	Participants were randomized to 1 of 3 groups: bilateral auricular acupuncture protocol at the Shenmen point ($n = 22$); bilateral auricular acupuncture at a "relaxation" point (a protocol believed to be effective against anxiety) ($n = 15$); or acupuncture at a sham acupuncture point ($n = 18$). Press-acupuncture needles were inserted at the respective auricular areas and remained in place for 48 h.
Outcomes	The Relaxation group was significantly less anxious at 30 min and at 24 and 48 h compared with the other 2 groups.
Wu et al. (36)	
Participants	35 healthy adult volunteers with preoperative anxiety.
Interventions	Participants received either auricular acupuncture at the Shenmen point, to a depth of about 0.2 cm ($n = 18$) or body acupuncture at 4 acupoints, to a depth of nearly 0.5 cm ($n = 17$). The acupuncture needles were 25–40 mm long and 0.25–0.30 mm in diameter. All subjects received twice-weekly treatments over 4 weeks (8 sessions in all). Each acupuncture session lasted approximately 30 min.
Outcomes	Scores on the Zung Self-Rating Anxiety Scale were significantly reduced from baseline in both groups.

(Continued)

TABLE 1 | Continued

Agelink et al. (37)	
Participants	36 patients with mild depression or anxiety disorder.
Interventions	Nine acupuncture sessions involving classical acupuncture points (He7, Pe6, Du20, Bl62, Ex6), or sham acupuncture (needles were applied epidermally at non-acupuncture points).
Outcomes	Compared with sham acupuncture, verum acupuncture was associated with a significant decrease in the mean resting heart rate and a significant decrease in the mean low LF:HF ratio.

TABLE 2 | Overview of characteristics of included studies evaluating acupuncture for schizophrenia.

Lee et al. (19)	
Participants	Chinese patients with schizophrenia.
Interventions	EA plus pharmacological therapy vs. sham EA plus pharmacological therapy. Acupuncture treatment vs. antipsychotics. Acupuncture plus antipsychotics vs. antipsychotics alone. Laser acupuncture vs. sham laser acupuncture.
Outcomes	In one study, EA plus drug therapy significantly improved auditory hallucinations and positive symptoms compared with sham EA plus drug therapy. In 4 studies, acupuncture significantly improved response rates compared with antipsychotics. In 7 studies, acupuncture plus antipsychotics significantly improved response rates compared with antipsychotics alone. One study reported that laser acupuncture ameliorated hallucination. Another study showed significant effects of laser acupuncture on response rates, BPRS and clinical global index scores compared with sham laser. The total number of RCTs included in the analysis, the total sample size, and methodological quality were too low to draw firm conclusions.
	Review paper
van den Noort et al. (16)	
Participants	1,181 patients with schizophrenia in regular care receiving acupuncture as add-on therapy.
Interventions	Only studies that used mechanical acupuncture in the treatment of patients with schizophrenia were included in this systematic review.
Outcomes	Most of the 26 identified studies had limited evidence for the use of acupuncture as add-on therapy in the treatment of positive, negative, and cognitive symptoms. Beneficial effects were reported for subjective and objective sleep measurements.
	Review paper
Ronan et al. (18)	
Participants	11 patients with schizophrenia.
Interventions	10-week course of individualized acupuncture treatment as an adjunct to routine care in schizophrenia.
Outcomes	All participants reported improvements in symptoms of schizophrenia, side effects of medication, energy, motivation, sleep, addictions, and other associated physical problems.
Bosch et al. (17)	
Participants	63-year-old woman with chronic schizophrenia.
Interventions	12 weekly acupuncture treatments, in addition to medication for chronic schizophrenia. Clinical diagnostic interviews and psychological testing (on sleep quality, depression, positive, and negative symptoms) were conducted before, immediately after and 3 months after the acupuncture treatment. The woman received individualized acupuncture treatment once weekly for 12 weeks, bilaterally for each point. The needles used were 0.25 × 25 or 0.20 × 15 mm stainless steel (depending on the place of needling) single-use needles and were placed according to TCM principles. After obtaining *de qi*, the needles were not stimulated thereafter, but left in place for 1 h.
Outcomes	The woman reported improved daily functioning and became less disturbed by her hallucinations. Her pain levels were also markedly reduced. Sleep improved immediately with acupuncture treatment. At 3 months after acupuncture treatment, positive and negative symptoms had decreased and depression scores had improved.
Bosch et al. (39)	
Participants	42-year-old man with chronic schizophrenia and co-morbid sleep disorders.
Interventions	In addition to his ongoing Western pharmacotherapy for schizophrenia, the man was treated for 12 weeks with acupuncture treatment in the clinic. The following acupuncture points were selected (with the absolute and the relative frequencies of use in parentheses) for use during the 12 weekly acupuncture treatments: Lidui (ST45) (12 = 100%); Sishencong (EX-HN 1) (11 = 92%); Zhaohai (Kl6) (10 = 83%); Shencang (Kl25) (7 = 58%); Baihui (DU20) (5 = 42%); Guanyuan (CV4) (4 = 33%); Zhiyin (BL67) (3 = 25%); Yutang (CV18) (3 = 25%); Wenliu (LI7) (2 = 17%); Taixi (Kl3) (1 = 8%); Lieque (LU7) (1 = 8%); Taiyang (EX-HN 5) (1 = 8%); Tianshu (ST25) (1 = 8%); Yingu (Kl10) (1 = 8%); Xiyan (eye of the knee) (1 = 8%); Shaofu (HT8) (1 = 8%).
Outcomes	The TCM diagnosis of a Liver Fire pattern before acupuncture was not as marked after the 12th acupuncture session. The patient had a small improvement in negative symptoms and in his general psychopathology, accompanied by a small reduction in the number of depressive symptoms. He experienced a marked improvement in sleep disorders following acupuncture, and actiwatch data revealed he was moving less during sleep.

As with the clinical evidence for acupuncture in depression and anxiety disorders, the clinical evidence is limited for the effectiveness of acupuncture as a treatment for schizophrenia. The meta-analysis performed by Lee and colleagues found that all of the included studies were limited by low methodological quality, the low overall number, and the small sample sizes (19). Moreover, as all of the studies were conducted in China, international trials are needed to investigate whether the effects can be replicated in other ethnicities.

The evidence consulted for schizophrenia is summarized in **Table 2**.

Acupuncture for the Treatment of Alzheimer's Disease

Another disorder that commonly exhibits neuropsychiatric symptoms is dementia. Neuropsychiatric symptoms are amongst the earliest signs and symptoms of neurocognitive disorders and incipient cognitive decline, and can be challenging to treat (40). Evidence shows that acupuncture can increase a patient's verbal and motor skills and improve mood and cognitive function. In an early Chinese trial, 38 patients with senile dementia were treated with acupuncture and acupoint-injection with aceglutamide (23). After treatment, symptoms had improved in 16 patients. In a small pilot study, 1 month of acupuncture treatment improved cognitive function in 8 patients with mild-to-moderate AD (22). Mini-Mental State Examination subscores assessing verbal orientation and motor coordination, as well as overall scores, were significantly improved from baseline. In a US study that included 11 patients with dementia (10 with AD and 1 with vascular dementia), depression and anxiety scores improved significantly after acupuncture treatment (21). These early pilot studies indicated that acupuncture treatment may be of benefit for neuropsychiatric symptoms in dementia, especially in AD.

More recently, a larger clinical trial involving 87 patients with mild-to-moderate AD reported that acupuncture may significantly improve cognitive function (20). When used as monotherapy, acupuncture treatment was associated with significantly greater decreases from baseline in Alzheimer's disease Assessment Scale-Cognitive (ADAS-cog) scores compared with donepezil. The improvement in cognitive function was observed for up to 12 weeks after the end of acupuncture treatment. Moreover, no patients discontinued treatment because of adverse events in the acupuncture group, but four patients did so in the donepezil group. In addition, a small-scale functional magnetic resonance imaging study explored the relationships between *de qi* sensations (a special needling sensation evoked by acupuncture) induced by different needling depths of acupuncture and their differential effects on the reorganizations of whole-brain networks in 12 patients with mild cognitive impairment (41). The results show that as compared with superficial needling, acupuncture with deep needling induces stronger, wider-ranging *de qi* sensations and enhances nodal centrality, primarily in the abnormal regions of the brain implicated in mild cognitive impairment. Hence, acupuncture treatment in mild-to-moderate AD appears to be not only beneficial but also safe, with the capacity to improve dysfunctional neural mechanisms involved in mild cognitive impairment.

The evidence consulted for AD is summarized in **Table 3**.

GLUTAMATE AND GLYCINE IN CNS DISORDERS

In the past decade, increasing evidence implicates the important role of glutamate in the pathophysiology of many CNS disorders (42, 43). L-Glutamate, the most common excitatory neurotransmitter in the CNS, is involved in synaptic plasticity and cognition (42, 44, 45). Glutamate receptors, synaptic receptors located primarily on the membranes of neuronal cells, are responsible for the glutamate-mediated postsynaptic excitation of neural cells and are important for neural communication, memory formation, learning, and regulation. Neural glutamate signaling is accommodated by two receptor families: ionotropic glutamate receptors (iGluRs; e.g., the N-methyl-D-aspartate [NMDA] receptor and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA] receptor) and metabotropic glutamate receptors (mGluRs) (46). The iGluRs produce excitatory glutamate-evoked currents, whereas the mGluRs are G protein-coupled receptors that control cellular processes through the G protein signaling cascades. Evidence implicates iGluRs, and/or mGluRs, as potential drug targets in neuropsychiatric disorders, including depression, anxiety, schizophrenia, and AD (46, 47).

One of the iGluRs is the NMDA receptor, which is activated by glutamate as well as glycine as a co-agonist. Its activation allows cations to flow through the cell membrane. The NMDA receptor is very important for controlling synaptic plasticity and memory function (48). Recent studies indicate that the dysregulation of the NMDA receptor may play an important role in schizophrenia (49, 50) and depression (51). Conversely, altered AMPA receptor functioning is a feature of many CNS disorders, including amyotrophic lateral sclerosis (ALS), ischemia, traumatic brain injury, epilepsies, and AD (47). The AMPA receptor is responsible for mediating most of the fast synaptic transmission in the CNS (47). Modulation of AMPA receptor numbers explains much of the plasticity of excitatory transmission in the brain, whereby increasing or decreasing AMPA receptors alters synaptic strength, which may be linked to neuropsychiatric disorders such as schizophrenia and depression (52, 53).

The release of glutamate in the synaptic cleft at a particular concentration is maintained by either glutamine synthetase or excitatory amino acid proteins, i.e., excitatory amino acid transporters (EAATs), which re-uptake excessive glutamate from the synapse. A high density of EAATs near the synapse ensures quick removal or transportation of any unbound glutamate. Of the five different membrane-bound transporters, EAAT2 performs more than 90% of the clearance of extracellular glutamate into crude synaptosomes to prevent neuronal excitotoxicity and hyperexcitability. When this process is impaired, it can allow large amounts of glutamate to spill out from the synapse, which may be a pathophysiological mechanism in CNS disorders (54) including schizophrenia, cognitive deficits, dementia and AD, and other disorders. Evidence suggests that EAAT2 activation is a promising therapeutic approach in many neuropsychiatric disease models (45).

Throughout the CNS, glycine acts as a co-agonist with L-glutamate at NMDA receptors. Glycine fluxes are regulated by two specific glycine transporters: GlyT1 and GlyT2. Whereas, GlyT2 is expressed in the spinal cord, brainstem and cerebellum, GlyT1 is expressed in these regions as well as in the forebrain areas such as the cortex, hippocampus,

TABLE 3 | Overview of characteristics of included studies evaluating acupuncture for Alzheimer's disease.

Chen (23)	
Participants	38 outpatients with senile dementia (SDAT, $n = 17$; MID, $n = 21$). A control group consisted of 20 elderly people (average age 69 years) with normal intelligence and mental functioning.
Interventions	Treatment with acupuncture and acupoint-injection with aceglutamide (1 ml, usually given after acupuncture). The acupuncture needles were retained in the selected acupoints for 20 min and were given every other day, for 3 courses of 15 sessions each. The acupoints chosen for needling were mainly in the Governor Vessel, such as Baihui (GV20), Naohu (GV17), Shuigou (GV26), etc., and aceglutamide was injected into tonic points, including Dazhui (GV14), Ganshu (BL18), Shenshu (BL23), Zusanli (ST36), etc. Acupoint injection generally followed acupuncture treatment.
Outcomes	After 45 sessions, improvement (improvement from baseline in HDS or FAQ scores and a general improvement in symptoms) was observed in 7 SDAT cases and 9 MID cases; treatment was excellent (HDS score increased by ≥2 grades or approached normal and FAQ score also approached normal) in 2 MID cases and effective (increase of 1 grade in HDS score and a considerable increase in FAQ score) in 7 MID cases.
Kao et al. (22)	
Participants	8 patients with mild-to-moderate AD.
Interventions	8 acupoints were selected according to the China National Standards on Acupoints (GB 12346-90): the Sishencong (Estra 6, 4 points on the scalp), Shenmen (HT7 on both wrists) and Taixi (KI3 on both feet). Needles were inserted to a depth of 0.5 inches at an angle into the Sishengcong, >0.5 inches directly into the Shenmen and 0.8 inches directly into the Taixi. Needling at each acupoint lasted for 30 min in total, comprising the needle testing and its re-insertion after every 10 min of needle therapy. Acupuncture was given in a 7-day treatment cycle with a 3-day break in-between for a total of 30 days.
Outcomes	Acupuncture was associated with significant improvements from baseline in cognition, as assessed by scores on the Mini Mental State Examination (MMSE) measuring verbal orientation ($p < 0.01$), motor coordination ($p < 0.05$) and overall score ($p < 0.05$). Acupuncture also produced a significant overall clinical improvement from baseline ($p < 0.05$) on the TCM Symptoms Checklist for AD.
Lombardo et al. (21)	
Participants	11 patients (10 with AD and 1 with vascular dementia).
Interventions	Patients received acupuncture twice weekly for 3 months; each patient had a minimum of 22 treatments. The initial 10 main acupoints selected were GB9, GV16, GV20, GV23, GV24, PC6, HT7, SP6, Sishencong, and Yintang. Secondary points selected included ST36, LI4, GB20, GV17, SP4, KI3, SI3, BL62, BL23, GV26, and the cervical and thoracic Huato Jiaji points.
Outcomes	Acupuncture was associated with statistically significant improvements in depression and anxiety scores. Some patients also experienced improvements in cognitive function.
Jia et al. (20)	
Participants	87 patients with mild-to-moderate AD.
Interventions	Acupuncture 3 times weekly for 12 weeks or once-daily donepezil 5 mg for 4 weeks then 10 mg/day for a further 8 weeks. No other treatments for AD were allowed during the study. Sterile, disposable needles (diameter, 0.25 mm; length, 40 mm) were used at the following acupoints: RN17 (danzhong), RN12 (zhongwan), RN6 (qihai), ST36 (zusanli), SJ5 (waiguan), and SP10 (xuehai). The following acupoints could be selected as auxiliary acupoints according to a patient's symptoms and tongue manifestation: LR3 (taichong), GB39 (xuanzhong), ST40 (fenglong), BL17 (geshu), ST44 (neiting), ST25 (tianshu), and RN4 (guan yu an). Except for RN17, RN12, RN6, and RN4, all other acupoints were bilateral. Acupuncture prescriptions were individualized to each patient, and different points were used based on the discretion of the acupuncturist. The acupuncture achieved *de qi*. To evoke needle sensation, the needles were inserted obliquely and upward 15 mm into RN17, 15–25 mm perpendicularly into RN12, RN6, and ST36, then rotated at small-amplitude and high frequency with a reinforcing method for 30 s. The needles were inserted perpendicularly 15–25 mm into SJ5, then rotated with normal reinforcement and normal reduction method for 30 s. For SP10, the needle was inserted obliquely 15–25 mm into the acupoint, then rotated with large-amplitude and low-frequency reducing method for 30 s. The needles remained in place for 30 min.
Outcomes	At 28 weeks, ADAS-cog scores were decreased from baseline by a significantly greater amount in the acupuncture group compared with the donepezil group. At weeks 10 and 28, mean CIBIC-Plus values were significantly lower in the acupuncture group vs. the donepezil group. No patients discontinued acupuncture treatment because of adverse events, whereas 4 donepezil recipients did so.
Bai et al. (41)	
Participants	12 patients with mild cognitive impairment and 12 age-match normal healthy controls.
Interventions	Each study group received 2 functional runs. They initially underwent a resting state scan for 6 min without any stimulation. Acupuncture was then performed at acupoint KI3 on the right leg (Taixi, located on the medial border of the foot posterior to the medial malleolus, in the depression between the tip of the medial malleolus and the Achilles tendon). The needle was inserted vertically to a depth of 1–2 cm with deep needling (DA), but of 1–2 mm in superficial needling (SA). Each acupuncture paradigm incorporated needle manipulation for 2 min, preceded by 1 min of rest and followed by 6 min of rest (no acupuncture manipulation). The presentation sequence of these 3 runs was randomized throughout the study population. Each participant performed only 1 run daily.
Outcomes	Compared with controls, patients exhibited losses of small-world attributes indicated by longer characteristic path lengths and larger clustering coefficients. Acupuncture with deep needling induced stronger and wider-ranging *de qi* sensations both in intensity and prevalence. Deep needling exhibited a modulatory effect to compensate the losses of small-world attributes in the patients; superficial needling had no such effect. Deep needling also enhanced nodal centrality, primarily in the abnormal regions of the brain of patients, including the hippocampus, postcentral cortex, and anterior cingulate cortex.

septum, and thalamus (55). GlyT2 is expressed by glycinergic nerve endings in rat spinal cord, while GlyT1 appears to be preferentially expressed by glial cells. Preclinical investigations suggest that GlyT2 is predominantly responsible for glycine uptake at glycinergic synapses, and that GlyT1 is involved in monitoring glycine concentration surrounding NMDA receptor-expressing synapses. In rats, GlyT1 inhibition potentiates NMDA receptor activity and affects NMDA receptor-dependent long-term potentiation (55). It is possible to modulate the function of the NMDA receptor by varying the availability of the glycine co-agonist. This has potential in the treatment of schizophrenia: the NMDA hypofunction hypothesis of schizophrenia postulates that increasing glutamatergic transmission via the NMDA receptors and inhibiting GlyT1 on glial cells enhances NMDA receptor neurotransmission by slowing the process of removal of glycine from the synapse and thus elevates synaptic glycine levels (55, 56).

Glutamate, Depression, and Anxiety

The monoamine hypothesis of depression contends that the underlying pathophysiology is due to depleted levels of serotonin, norepinephrine, and/or dopamine in the CNS (57). However, no direct evidence supports a primary dysfunction of a specific monoamine system in patients with MDDs. Moreover, not only do many patients fail to respond to monoamine antidepressants, but residual symptoms, relapses and recurrences are common even with adequate dosing of these medications (58) and it can take up to several days or weeks for core depressive symptoms to begin to lift after monoamine antidepressants have elevated synaptic monoamine levels (57–59). MDD pathogenesis appears to involve something else beyond the monoamine system.

Glutamatergic modulation shows potential in antidepressant treatment. A single subanesthetic intravenous dose of the NMDA receptor antagonist ketamine acts rapidly in treatment-resistant depression within hours of administration, with effects that are typically sustained for 7–14 days (60). Moreover, the ketamine metabolite (2R,6R)-hydroxynorketamine [(2R,6R)-HNK] appears to have the antidepressant effects of ketamine and lacks the psychiatric, psychotomimetic, cardiovascular, neurological, and other side effects associated with acute dosing of ketamine in patients with depression (52). Unlike ketamine, (2R,6R)-HNK is not a NMDA receptor antagonist but is associated with the modulation of the AMPA receptor. Thus, both NMDA and AMPA receptors may be involved in the pathophysiological changes of depression and potentially represent new targets for the development of rapid-acting antidepressants.

The glutamatergic system also plays a major role in the pathogenesis of anxiety (61–63). Long-term administration of various antidepressant agents including selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs) lowers glutamatergic activity in some regions, such as the hippocampus (61, 64), while the acute administration of NMDA receptor antagonists produces anti-anxiety and antidepressant effects in preclinical and clinical models (43, 62). Lamotrigine, used in epilepsy and bipolar depression, inhibits glutamate release and has proven efficacy in certain symptoms of post-traumatic stress disorder (PTSD), namely, re-experiencing and avoidance/numbing (65). Similarly, topiramate, which acts partly as an AMPA/kainate blocker, decreases re-experiencing symptoms in PTSD (66).

Glutamate Receptor Dysregulation Contributes to Schizophrenia

Animal models of schizophrenia have implicated glutamate receptor dysregulation and other proteins relating to glutamate transmission, including EAAT2 (54). The glutamate hypothesis of schizophrenia is supported by the finding that the NMDA receptor antagonist phencyclidine blocks glutamate-activated postsynaptic currents and induces schizophrenia-like symptoms, including psychosis and cognitive impairment (50). Moreover, phencyclidine impairs prepulse inhibition (PPI) of the startle reflex, a simple form of information processing that is consistently reduced in schizophrenia. In rats treated with ceftriaxone and acute phencyclidine, PPI was more impaired compared with rats given either treatment alone (67). A broad-spectrum cephalosporin antibiotic, ceftriaxone stimulates EAAT2 expression and lessens neurotoxicity by inhibiting neuronal cell death associated with glutamate excitotoxicity (68). Upregulation of EAAT2 expression by ceftriaxone is thought to be via presynaptic activation of the mGluR, as mGluR2/3 agonist treatment prevented the PPI impairment associated with ceftriaxone-induced upregulation of EAAT2 in rats (69). EAAT2 overexpression causing PPI impairment may be due to glutamate spillover, which mGluR2/3 relies on for activation due to its perisynaptic localization (54). In patients with schizophrenia, oral clozapine 25–35 mg/kg/day for 3 weeks downregulated astrocytic EAAT2 levels and increased extracellular glutamate levels (70).

Interestingly, expression of the presynaptic protein synaptophysin, which is involved in neurotransmitter release, is significantly increased in the same anatomical areas where EAAT2 levels are downregulated by clozapine (71). This suggests that glutamate release may assist clozapine in potentiation of the excitatory synapse. More support for glutamate receptor dysregulation in schizophrenia is seen with the selective GlyT1 inhibitor sarcosine (N-methylglycine), which has shown promise in the treatment of cognitive impairment in patients with chronic schizophrenia (56, 72).

The Dysregulated Glutamate System and AD

It is well-known that AD is associated with reductions in glutamate transporter capacity and protein expression, as well as a selective loss of the vesicular glutamate transporter (73–75). Recent evidence further points to impairment of EAAT2 function in AD, with findings of significantly lower levels of EAAT2 gene expression in the cortex and decreased EAAT2 immunoreactivity in the motor cortex of patients with AD

(76). Although EAAT2b expression did not vary significantly according to disease severity, significantly upregulated levels of exon-skipping variant mRNA expression have been found, which reduces wild-type EAAT2 protein expression in primary astrocytes and inhibits glutamate transport (76). The correlation of EAAT2 expression with increasing neurodegeneration, in combination with the ability of exon-skipping variants to reduce glutamate reuptake, suggests that increased glutamate levels may propagate excitotoxic processes implicated in AD pathogenesis.

Moreover, iGluRs may also contribute to the excitotoxic processes implicated in AD pathogenesis. It is well-established that the NMDA receptor plays an important role in excitotoxicity (77). Hyperactivity of the NMDA receptor may result in a flooding of cations (e.g., Ca^{2+}) into the neuron leading to degeneration of the dendritic spines or even the death of the neuron. In patients with AD, treatment with the uncompetitive NMDA receptor antagonist memantine can benefit the cognitive symptoms of AD (78). Notably, in preclinical models of AD, AMPA-mediated transmission, and altered synapse morphology correlated with cognitive decline (47). An initial loss of dendritic spines and synapses is accompanied by a concomitant increase in presynaptic release probability, as the neuronal circuit attempts to compensate for synaptic dysfunction and loss (47). This evidence suggests that increased synaptic glutamate levels induced by a reduction in reuptake may trigger iGluR hyperactivity and lead to the excitotoxic processes relating to the cognitive symptoms of AD.

ACUPUNCTURE MODULATES GLUTAMATE NEUROTRANSMISSION

To date, very little neuroscience research has explored the effects of acupuncture on the role of glutamate in neuropsychiatric disorders. While studies of acupuncture analgesia indicate that acupuncture stimulation may modulate levels of expression of glutamate expression and its receptor, as well as EAAT expression (79, 80), no existing studies have reported on acupuncture-induced modulation of the glycine transporter or other upstream regulatory mechanisms (e.g., D-amino acid oxidase and the amino acid transporter system).

Research has reported that both high- and low-frequency EA significantly decreases upregulated levels of NMDA receptor 1 and 2A and AMPA receptor 1 expression in the spinal cord in an inflammatory pain animal model (81). These findings are corroborated by other research reporting that EA (10 Hz) inhibited phosphorylation of NMDA receptor 1 in spinal cord and alleviated pain in a rat model of inflammatory pain (82). At the supraspinal level, alternating high- and low-frequency EA decreased levels of NMDA receptor 1 and c-fos expression in the rostral ventromedial medulla in an animal model of visceral pain (83). Thus, the expression of glutamate and NMDA receptors may be modulated by acupuncture stimulation in the CNS.

Acupuncture also modulates EAAT2 expression. A recent study examined the effect of acupuncture on depressive behaviors and EAAT2 in rats subjected to chronic unpredictable mild stress (44). Both acupuncture therapy and drug treatment with the glutamate reuptake enhancer riluzole significantly increased sucrose consumption in the sucrose preference test paradigm. This increase in sucrose consumption was associated with an elevated food intake and shortened latency in the novelty-suppressed feeding test paradigm. The amelioration of depressive behavioral actions was consistent with increasing numbers of EAAT2-positive cells and protein expression in the hippocampus and prefrontal cortex. EAAT2 mRNA expression was also increased in the prefrontal cortex, but there was no change in the hippocampus. Moreover, the antidepressant effect was observed later with acupuncture than with riluzole, indicating that repeated acupuncture stimulation may be needed to accumulate EAAT2 expression. Thus, acupuncture-mediated modulation of EAAT2 expression may ameliorate depression.

CONCLUSIONS

In summary, evidence indicates that acupuncture treatment may be of benefit in several neuropsychiatric disorders, including depression, anxiety, schizophrenia, and AD. The pathophysiology of these disorders may be associated with glutamate dysregulation, marked by a high rate of glutamate release and elevated expression of glutamate receptors and glutamate transporters in the CNS. The ability of acupuncture stimulation to modulate glutamate receptor and EAAT expression suggests that the treatment effects of acupuncture are underpinned by its intervention in the dysregulated glutamate system. Further preclinical and clinical studies are needed to clarify the possible mechanisms of acupuncture in these neuropsychiatric disorders and to establish protocols for treatment guidelines.

AUTHOR CONTRIBUTIONS

C-HT and IM: collecting and analyzing literature and writing the manuscript; Y-HC: designing and coordinating the study as well as writing the manuscript.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Science and Technology, Taiwan (MOST 107-2320-B-039—031 -MY3, 106-2320-B-039—019, 107-2314-B-039-058-MY2, and 106-2314-B-039-012) and China Medical University (CMU 105-S-25). This work was also supported by the Chinese Medicine Research Center, China Medical University from the Featured Areas Research Center Program within the framework of the Higher Education Sprout Project under the Ministry of Education (MOE) in Taiwan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Stein DJ, Phillips KA, Bolton D, Fulford KW, Sadler JZ, Kendler KS. What is a mental/psychiatric disorder? From DSM-IV to DSM-V. *Psychol Med.* (2010) 40:1759–65. doi: 10.1017/S0033291709992261
2. Juul SH, Nemeroff CB. Psychiatric epidemiology. *Handb Clin Neurol.* (2012) 106:167–89. doi: 10.1016/B978-0-444-52002-9.00010-3
3. Park-Lee E, Lipari RN, Hedden SL, Kroutil LA, Porter JD. *Receipt of Services for Substance Use Andmental Health Issues Among Adults: Results From the 2016 National Survey on Drug Use and Health CBHSQ Data Review.* Rockville, MD: Substance Abuse and Mental Health Services Administration (2012–2017).
4. Substance Abuse and Mental Health Services Administration (SAMHSA). *Mental Health and Substance Use Disorders.* Rockville, MD: Substance Abuse and Mental Health Services Administration (2017).
5. Wittchen HU, Jacobi F. Size and burden of mental disorders in Europe–a critical review and appraisal of 27 studies. *Eur Neuropsychopharmacol.* (2005) 15:357–76. doi: 10.1016/j.euroneuro.2005.04.012
6. World Health Organization Regional Office for Europe. Prevalence of Mental Disorders. World Health Organization Regional Office for Europe (2018).
7. Clevenger SS, Malhotra D, Dang J, Vanle B, IsHak WW. The role of selective serotonin reuptake inhibitors in preventing relapse of major depressive disorder. *Ther Adv Psychopharmacol.* (2018) 8:49–58. doi: 10.1177/2045125317737264
8. Winsky L, Driscoll J, Brady L. CHAPTER 2 - drug discovery and development initiatives at the National Institute of Mental Health: from cell-based systems to proof of concept. In: McArthur RA and Borsini F, Editors. Animal and Translational Models for CNS Drug Discovery. San Diego, CA: Academic Press (2008). p. 59–74.
9. Zhao ZQ. Neural mechanism underlying acupuncture analgesia. *Prog Neurobiol.* (2008) 85:355–75. doi: 10.1016/j.pneurobio.2008.05.004
10. Smith CA, Armour M, Lee MS, Wang LQ, Hay PJ. Acupuncture for depression. *Cochrane Database Syst Rev.* (2018) 3:CD004046. doi: 10.1002/14651858.CD004046.pub4
11. Dong B, Chen Z, Yin X, Li D, Ma J, Yin P, et al. The efficacy of acupuncture for treating depression-related insomnia compared with a control group: a systematic review and meta-analysis. *Biomed Res Int.* (2017) 2017:9614810. doi: 10.1155/2017/9614810
12. Fan L, Gong J, Fu W, Chen Z, Xu N, Liu J, et al. Gender-related differences in outcomes on acupuncture and moxibustion treatment among depression patients. *J Altern Complement Med.* (2015) 21:673–80. doi: 10.1089/acm.2015.0068
13. Fan L, Fu W-B, Xu N-G, Liu J-H, Li Z-P, Ou A-H. [Impacts of acupuncture and moxibustion on outcome indices of depression patients' subjective reports]. *World J Acupunct Moxibust.* (2013) 23:22–8.
14. Errington-Evans N., Acupuncture for anxiety. *CNS Neurosci Ther.* (2012) 18:277–84. doi: 10.1111/j.1755-5949.2011.00254.x
15. Spence DW, Kayumov L, Chen A, Lowe A, Jain U, Katzman MA, et al. Acupuncture increases nocturnal melatonin secretion and reduces insomnia and anxiety: a preliminary report. *J Neuropsychiatry Clin Neurosci.* (2004) 16:19–28. doi: 10.1176/jnp.16.1.19
16. van den Noort M, Yeo S, Lim S, Lee SH, Staudte H, Bosch P. Acupuncture as add-on treatment of the positive, negative, and cognitive symptoms of patients with schizophrenia: a systematic review. *Medicines* (2018) 5:29. doi: 10.3390/MEDICINES5020029
17. Bosch P, Staudte H, van den Noort M, Lim S. A case study on acupuncture in the treatment of schizophrenia. *Acupunct Med.* (2014) 32:286–9. doi: 10.1136/acupmed-2014-010547
18. Ronan P, Robinson N, Harbinson D, Macinnes D. A case study exploration of the value of acupuncture as an adjunct treatment for patients diagnosed with schizophrenia: results and future study design. *Zhong Xi Yi Jie He Xue Bao* (2011) 9:503–14. doi: 10.3736/jcim20110507
19. Lee MS, Shin BC, Ronan P, Ernst E. Acupuncture for schizophrenia: a systematic review and meta-analysis. *Int J Clin Pract.* (2009) 63:1622–33. doi: 10.1111/j.1742-1241.2009.02167.x
20. Jia Y, Zhang X, Yu J, Han J, Yu T, Shi J, et al. Acupuncture for patients with mild to moderate Alzheimer's disease: a randomized controlled trial. *BMC Complement Altern Med.* (2017) 17:556. doi: 10.1186/s12906-017-2064-x
21. Lombardo NBE, Dresser MVB, Malivert M, McManus CA, Vehvilainen L, Ooi WL, et al. Acupuncture as treatment for an21 doixiety and depression in persons with dementia: results of a feasibility and effectiveness study. *Alzheimers Care Today* (2001) 2:28–41. doi: 10.1016/j.ctim.2007.07.005
22. Kao H, Wang M, Yu S, Yuan S, Mao W, Zhang W, et al. Acupuncture enhancement in clinical symptoms and cognitive motor abilities of the Alzheimer's disease patients. *Neurobiol Aging* (2000) 21:79 doi: 10.1016/S0197-4580(00)82578-6
23. Chen Y. Clinical research on treating senile dementia by combining acupuncture with acupoint-injection. *Acupunct Electrother Res.* (1992) 17:61–73. doi: 10.3727/036012992816357800
24. Lao L, Hamilton GR, Fu J, Berman BM. Is acupuncture safe? A systematic review of case reports. *Altern Ther Health Med.* (2003) 9:72–83.
25. NIH Consensus Conference. Acupuncture. *JAMA* (1998) 280:1518–24.
26. White A. A cumulative review of the range and incidence of significant adverse events associated with acupuncture. *Acupunct Med.* (2004) 22:122–33. doi: 10.1136/aim.22.3.122
27. Yang C, Hao Z, Zhang LL, Guo Q. Efficacy and safety of acupuncture in children: an overview of systematic reviews. *Pediatr Res.* (2015) 78:112–9. doi: 10.1038/pr.2015.91
28. He W, Zhao X, Li Y, Xi Q, Guo Y. Adverse events following acupuncture: a systematic review of the Chinese literature for the years 1956-2010. *J Altern Complement Med.* (2012) 18:892–901. doi: 10.1089/acm.2011.0825
29. Witt CM, Pach D, Brinkhaus B, Wruck K, Tag B, Mank S, et al. Safety of acupuncture: results of a prospective observational study with 229,230 patients and introduction of a medical information and consent form. *Forsch Komplementmed.* (2009) 16:91–7. doi: 10.1159/000209315
30. Zhao L, Zhang FW, Li Y, Wu X, Zheng H, Cheng LH, et al. Adverse events associated with acupuncture: three multicentre randomized controlled trials of 1968 cases in China. *Trials* (2011) 12:87. doi: 10.1186/1745-6215-12-87
31. Wu J, Yeung AS, Schnyer R, Wang Y, Mischoulon D. Acupuncture for depression: a review of clinical applications. *Can J Psychiatry* (2012) 57:397–405. doi: 10.1177/070674371205700702
32. Zhang ZJ, Chen HY, Yip KC, Ng R, Wong VT. The effectiveness and safety of acupuncture therapy in depressive disorders: systematic review and meta-analysis. *J Affect Disord.* (2010) 124:9–21. doi: 10.1016/j.jad.2009.07.005
33. Chung KF, Yeung WF, Yu YM, Yung KP, Zhang SP, Zhang ZJ, et al. Acupuncture for residual insomnia associated with major depressive disorder: a placebo- and sham-controlled, subject- and assessor-blind, randomized trial. *J Clin Psychiatry* (2015) 76:e752–60. doi: 10.4088/JCP.14m09124
34. British Acupuncture Council, Acupuncture Research Resource Council. *Depression, Anxiety and Acupuncture: The Evidence for Effectiveness.* Briefing paper No. 9 (2002).
35. Wang SM, Kain ZN. Auricular acupuncture: a potential treatment for anxiety. *Anesth Analg.* (2001) 92:548–53. doi: 10.1213/00000539-200102000-00049
36. Wu S, Liang J, Zhu X, Liu X, Miao D. Comparing the treatment effectiveness of body acupuncture and auricular acupuncture in preoperative anxiety treatment. *J Res Med Sci.* (2011) 16:39–42.
37. Agelink MW, Sanner D, Eich H, Pach J, Bertling R, Lemmer W, et al. [Does acupuncture influence the cardiac autonomic nervous system in patients with minor depression or anxiety disorders?]. *Fortschr Neurol Psychiatr.* (2003) 71:141–9. doi: 10.1055/s-2003-37756
38. Pilkington K. Anxiety, depression and acupuncture: a review of the clinical research. *Auton Neurosci.* (2010) 157:91–5. doi: 10.1016/j.autneu.2010.04.002
39. Bosch P, Staudte H, Yeo SJ, Lee SH, Lim S, van den Noort M. Acupuncture treatment of a male patient suffering from long-term schizophrenia and sleep disorders. *J Tradit Chin Med.* (2017) 37:862–7. doi: 10.1016/S0254-6272(18)30052-9
40. Lanctot KL, Amatniek J, Ancoli-Israel S, Arnold SE, Ballard C, Cohen-Mansfield J, et al. Neuropsychiatric signs and symptoms of Alzheimer's disease: new treatment paradigms. *Alzheimers Dement.* (2017) 3:440–9. doi: 10.1016/j.trci.2017.07.001
41. Bai L, Zhang M, Chen S, Ai L, Xu M, Wang D, et al. Characterizing acupuncture *de qi* in mild cognitive impairment: relations with small-world efficiency of functional brain networks. *Evid Based Complement Alternat Med.* (2013) 2013:304804. doi: 10.1155/2013/304804
42. Soni N, Reddy BV, Kumar P. GLT-1 transporter: an effective pharmacological target for various neurological disorders. *Pharmacol Biochem Behav.* (2014) 127:70–81. doi: 10.1016/j.pbb.2014.10.001
43. Pilc A, Wieronska JM, Skolnick P. Glutamate-based antidepressants: preclinical psychopharmacology. *Biol Psychiatry* (2013) 73:1125–32. doi: 10.1016/j.biopsych.2013.01.021

44. Luo D, Ma R, Wu Y, Zhang X, Liu Y, Wang L, et al. Mechanism underlying acupuncture-ameliorated depressive behaviors by enhancing glial glutamate transporter in Chronic Unpredictable Mild Stress (CUMS) rats. *Med Sci Monit*. (2017) 23:3080–7. doi: 10.12659/MSM.902549
45. Takahashi K, Foster JB, Lin CL. Glutamate transporter EAAT2: regulation, function, and potential as a therapeutic target for neurological and psychiatric disease. *Cell Mol Life Sci*. (2015) 72:3489–506. doi: 10.1007/s00018-015-1937-8
46. Reiner A, Levitz J. Glutamatergic signaling in the central nervous system: ionotropic and metabotropic receptors in concert. *Neuron* (2018) 98:1080–98. doi: 10.1016/j.neuron.2018.05.018
47. Chang PK, Verbich D, McKinney RA. AMPA receptors as drug targets in neurological disease–advantages, caveats, and future outlook. *Eur J Neurosci*. (2012) 35:1908–16. doi: 10.1111/j.1460-9568.2012.08165.x
48. Newcomer JW, Farber NB, Olney JW. NMDA receptor function, memory, and brain aging. *Dialogues Clin Neurosci*. (2000) 2:219–32.
49. Lin CH, Lane HY, Tsai GE. Glutamate signaling in the pathophysiology and therapy of schizophrenia. *Pharmacol Biochem Behav*. (2012) 100:665–77. doi: 10.1016/j.pbb.2011.03.023
50. Javitt DC. Glutamate and schizophrenia: phencyclidine, N-methyl-D-aspartate receptors, and dopamine-glutamate interactions. *Int Rev Neurobiol*. (2007) 78:69–108. doi: 10.1016/S0074-7742(06)78003-5
51. Serafini G, Howland RH, Rovedi F, Girardi P, Amore M. The role of ketamine in treatment-resistant depression: a systematic review. *Curr Neuropharmacol*. (2014) 12:444–61. doi: 10.2174/1570159X12666140619204251
52. Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, et al. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature* (2016) 533:481–6. doi: 10.1038/nature17998
53. Rubio MD, Drummond JB, Meador-Woodruff JH. Glutamate receptor abnormalities in schizophrenia: implications for innovative treatments. *Biomol Ther*. (2012) 20:1–18. doi: 10.4062/biomolther.2012.20.1.001
54. O'Donovan SM, Sullivan CR, McCullumsmith RE. The role of glutamate transporters in the pathophysiology of neuropsychiatric disorders. *npj Schizophrenia* (2017) 3:32. doi: 10.1038/s41537-017-0037-1
55. Abdel-Magid AF. Glycine transporter 1 inhibitors and the promise of a new schizophrenia therapy. *ACS Med Chem Lett*. (2016) 7:549–51. doi: 10.1021/acsmedchemlett.6b00183
56. Hashimoto K. Glycine transport inhibitors for the treatment of schizophrenia. *Open Med Chem J*. (2010) 4:10–9. doi: 10.2174/1874104501004010010
57. Delgado PL. Depression: the case for a monoamine deficiency. *J Clin Psychiatry* (2000) 61(Suppl. 6):7–11.
58. Pomara N, Sidtis JJ. Brain neurotoxic amyloid-beta peptides: their potential role in the pathophysiology of depression and as molecular therapeutic targets. *Br J Pharmacol*. (2010) 161:768–70. doi: 10.1111/j.1476-5381.2010.00948.x
59. Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. *Arch Gen Psychiatry* (2007) 64:327–37. doi: 10.1001/archpsyc.64.3.327
60. Ionescu DF, Papakostas GI. Experimental medication treatment approaches for depression. *Transl Psychiatry* (2017) 7:e1068. doi: 10.1038/tp.2017.33
61. Riaza Bermudo-Soriano C, Perez-Rodriguez MM, Vaquero-Lorenzo C, Baca-Garcia E. New perspectives in glutamate and anxiety. *Pharmacol Biochem Behav*. (2012) 100:752–74. doi: 10.1016/j.pbb.2011.04.010
62. Garakani A, Mathew SJ, Charney DS. Neurobiology of anxiety disorders and implications for treatment. *Mt Sinai J Med*. (2006) 73:941–9.
63. Bergink V, van Megen HJ, Westenberg HG. Glutamate and anxiety. *Eur Neuropsychopharmacol*. (2004) 14:175–83. doi: 10.1016/S0924-977X(03)00100-7
64. Bonanno G, Giambelli R, Raiteri L, Tiraboschi E, Zappettini S, Musazzi L, et al. Chronic antidepressants reduce depolarization-evoked glutamate release and protein interactions favoring formation of SNARE complex in hippocampus. *J Neurosci*. (2005) 25:3270–9. doi: 10.1523/JNEUROSCI.5033-04.2005
65. Zavodnick AD, Ali R. Lamotrigine in the treatment of unipolar depression with and without comorbidities: a literature review. *Psychiatr Q*. (2012) 83:371–83. doi: 10.1007/s11126-012-9208-4
66. Berlant J, van Kammen DP. Open-label topiramate as primary or adjunctive therapy in chronic civilian posttraumatic stress disorder: a preliminary report. *J Clin Psychiatry* (2002) 63:15–20. doi: 10.4088/JCP.v63n0104
67. Melone M, Bellesi M, Gubbini A, Conti F. GLT-1 up-regulation enhances the effect of PCP on prepulse inhibition of the startle reflex in adult rats. *Schizophr Res*. (2009) 109:196–7. doi: 10.1016/j.schres.2009.02.010
68. Lee SG, Su ZZ, Emdad L, Gupta P, Sarkar D, Borjabad A, et al. Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. *J Biol Chem*. (2008) 283:13116–23. doi: 10.1074/jbc.M707697200
69. Bellesi M, Conti F. The mGluR2/3 agonist LY379268 blocks the effects of GLT-1 upregulation on prepulse inhibition of the startle reflex in adult rats. *Neuropsychopharmacology* (2010) 35:1253–60. doi: 10.1038/npp.2009.225
70. Melone M, Vitellaro-Zuccarello L, Vallejo-Illarramendi A, Pérez-Samartin A, Matute C, Cozzi A, et al. The expression of glutamate transporter GLT-1 in the rat cerebral cortex is down-regulated by the antipsychotic drug clozapine. *Mol Psychiatry* (2001) 6:380. doi: 10.1038/sj.mp.4000880
71. Bragina L, Melone M, Fattorini G, Torres-Ramos M, Vallejo-Illarramendi A, Matute C, et al. GLT-1 down-regulation induced by clozapine in rat frontal cortex is associated with synaptophysin up-regulation. *J Neurochem*. (2006) 99:134–41. doi: 10.1111/j.1471-4159.2006.04030.x
72. Lin CY, Liang SY, Chang YC, Ting SY, Kao CL, Wu YH, et al. Adjunctive sarcosine plus benzoate improved cognitive function in chronic schizophrenia patients with constant clinical symptoms: a randomised, double-blind, placebo-controlled trial. *World J Biol Psychiatry* (2017) 18:357–68. doi: 10.3109/15622975.2015.1117654
73. Kirvell SL, Esiri M, Francis PT. Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease. *J Neurochem*. (2006) 98:939–50. doi: 10.1111/j.1471-4159.2006.03935.x
74. Li S, Mallory M, Alford M, Tanaka S, Masliah E. Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. *J Neuropathol Exp Neurol*. (1997) 56:901–11. doi: 10.1097/00005072-199708000-00008
75. Masliah E, Alford M, DeTeresa R, Mallory M, Hansen L. Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. *Ann Neurol*. (1996) 40:759–66. doi: 10.1002/ana.410400512
76. Scott HA, Gebhardt FM, Mitrovic AD, Vandenberg RJ, Dodd PR. Glutamate transporter variants reduce glutamate uptake in Alzheimer's disease. *Neurobiol Aging* (2011) 32:553 e1–11. doi: 10.1016/j.neurobiolaging.2010.03.008
77. Rothstein JD. Excitotoxicity hypothesis. *Neurology* (1996) 47:S19–25; Discussion S26.
78. Parsons CG, Stoffler A, Danysz W. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system–too little activation is bad, too much is even worse. *Neuropharmacology* (2007) 53:699–723. doi: 10.1016/j.neuropharm.2007.07.013
79. Cui L, Ding Y, Zeng J, Feng Y, Li M, Ding M. Spinal glutamate transporters are involved in the development of electroacupuncture tolerance. *Int J Mol Sci*. (2016) 17:357. doi: 10.3390/ijms17030357
80. Gao P, Gao XI, Fu T, Xu D, Wen Q. Acupuncture: emerging evidence for its use as an analgesic (Review). *Exp Ther Med*. (2015) 9:1577–81. doi: 10.3892/etm.2015.2348
81. Choi BT, Kang J, Jo UB. Effects of electroacupuncture with different frequencies on spinal ionotropic glutamate receptor expression in complete Freund's adjuvant-injected rat. *Acta Histochem*. (2005) 107:67–76. doi: 10.1016/j.acthis.2004.07.008
82. Meng XZ, Lao LX, Shen XY, Berman BM, Ren K, Wei PK, et al. Electroacupuncture inhibits spinal interleukin-17A to alleviate inflammatory pain in a rat model. *Open Pain J*. (2013) 6:183–9. doi: 10.2174/1876386320130624001
83. Qi DB, Li WM. Effects of electroacupuncture on expression of c-fos protein and N-methyl-D-aspartate receptor 1 in the rostral ventromedial medulla of rats with chronic visceral hyperalgesia. *Zhong Xi Yi Jie He Xue Bao* (2012) 10:416–23. doi: 10.3736/jcim20120410

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 14 February 2019
doi: 10.3389/fpsyt.2019.00049

Targeting mGlu Receptors for Optimization of Antipsychotic Activity and Disease-Modifying Effect in Schizophrenia

Ferdinando Nicoletti[1,2]*, *Rosamaria Orlando*[1], *Luisa Di Menna*[2], *Milena Cannella*[2], *Serena Notartomaso*[2], *Giada Mascio*[2], *Luisa Iacovelli*[1], *Francesco Matrisciano*[3], *Francesco Fazio*[2], *Filippo Caraci*[4,5], *Agata Copani*[4,6], *Giuseppe Battaglia*[2] and *Valeria Bruno*[1,2]

[1] Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy, [2] IRCCS Neuromed, Pozzilli, Italy, [3] Department of Psychiatry, The Psychiatric Institute, College of Medicine, University of Illinois, Chicago, IL, United States, [4] Department of Drug Sciences, University of Catania, Catania, Italy, [5] Oasi Research Institute (IRCCS), Troina, Italy, [6] Institute of Biostructure and Bioimaging, National Research Council, Catania, Italy

OPEN ACCESS

Edited by:
Hsien-Yuan Lane,
China Medical University, Taiwan

Reviewed by:
Joshua T. Kantrowitz,
Columbia University, United States
Alessandro Usiello,
Second University of Naples, Italy

***Correspondence:**
Ferdinando Nicoletti
ferdinandonicoletti@hotmail.com

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: 13 November 2018
Accepted: 23 January 2019
Published: 14 February 2019

Citation:
Nicoletti F, Orlando R, Di Menna L, Cannella M, Notartomaso S, Mascio G, Iacovelli L, Matrisciano F, Fazio F, Caraci F, Copani A, Battaglia G and Bruno V (2019) Targeting mGlu Receptors for Optimization of Antipsychotic Activity and Disease-Modifying Effect in Schizophrenia. Front. Psychiatry 10:49. doi: 10.3389/fpsyt.2019.00049

Metabotropic glutamate (mGlu) receptors are considered as candidate drug targets for the treatment of schizophrenia. These receptors form a family of eight subtypes (mGlu1 to −8), of which mGlu1 and −5 are coupled to $G_{q/11}$, and all other subtypes are coupled to $G_{i/o}$. Here, we discuss the possibility that selective ligands of individual mGlu receptor subtypes may be effective in controlling the core symptoms of schizophrenia, and, in some cases, may impact mechanisms underlying the progression of the disorder. Recent evidence indicates that activation of mGlu1 receptors inhibits dopamine release in the meso-striatal system. Hence, selective positive allosteric modulators (PAMs) of mGlu1 receptors hold promise for the treatment of positive symptoms of schizophrenia. mGlu5 receptors are widely expressed in the CNS and regulate the activity of cells that are involved in the pathophysiology of schizophrenia, such as cortical GABAergic interneurons and microglial cells. mGlu5 receptor PAMs are under development for the treatment of schizophrenia and cater the potential to act as disease modifiers by restraining neuroinflammation. mGlu2 receptors have attracted considerable interest because they negatively modulate 5-HT_{2A} serotonin receptor signaling in the cerebral cortex. Both mGlu2 receptor PAMs and orthosteric mGlu2/3 receptor agonists display antipsychotic-like activity in animal models, and the latter drugs are inactive in mice lacking mGlu2 receptors. So far, mGlu3 receptors have been left apart as drug targets for schizophrenia. However, activation of mGlu3 receptors boosts mGlu5 receptor signaling, supports neuronal survival, and drives microglial cells toward an antiinflammatory phenotype. This strongly encourages research of mGlu3 receptors in schizophrenia. Finally, preclical studies suggest that mGlu4 receptors might be targeted by novel antipsychotic drugs, whereas studies of mGlu7 and mGlu8 receptors in animal models of psychosis are still at their infancy.

Keywords: metabotropic glutamate receptors, schizophrenia, positive allosteric modulator, development of cortical interneurons, receptor cross-talk

BACKGROUND

In spite of the continuous development of "monoaminergic" antipsychotic agents, drug treatment of schizophrenia remains suboptimal. Current second-generation antipsychotic drugs include drugs with different affinity for dopamine and serotonin receptor subtypes, such as clozapine, olanzapine, quetiapine, asenapine, ziprasidone, lurasidone, risperidone, paliperidone, iloperidone, aripiprazole, cariprazine, and brexpiprazole. These drugs display good therapeutic efficacy against positive symptoms of schizophrenia and their use may slow the progression of brain atrophy (as opposed to first generation antipsychotics) (1). However, a significant proportion of patients affected by schizophrenia is refractory to medication, and a prophylactic use of these agents (i.e., their use prior to the first episode of psychosis) is limited by class-related adverse effects, such as sedation, weight gain, and anticholinergic effects. In addition, none of the first- or second-generation antipsychotics is effective in improving cognitive dysfunction associated with schizophrenia, with the possible exception of clozapine, which is considered as a second- or third-line drug because of serious safety concerns.

Thus, there is an urgent need for new safer antipsychotic agents acting at new targets that lie at the core of the pathophysiology of schizophrenia. A new Molecular Psychiatry article entitled "mGluR5 hypofunction is integral to glutamatergic dysregulation in schizophrenia" (2) is one point of arrival of years of extensive research linking metabotropic glutamate (mGlu) receptors to the pathophysiology and treatment of schizophrenia. This article shows that mGlu5 receptor signaling is blunted in the dorsolateral prefrontal cortex (DLPFC) of individuals affected by schizophrenia (2).

mGlu5 is one of the eight mGlu receptor subtypes that are traditionally subdivided into three groups on the basis of their amino acid sequence, pharmacological profile, and signal transduction mechanisms in heterologous expression systems. Group I includes mGlu1 and mGlu5 receptors, which are coupled to $G_{q/11}$ proteins. Group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7, and mGlu8) mGlu receptors are all coupled to Gi/o proteins (3). This classification is universally accepted but incomplete from a functional standpoint. Native mGlu receptors activate multiple transduction pathways in a cell-and context-dependent fashion, and numerous subtype-selective biased ligands have been developed. For example, mGlu5 receptors are physically and functionally linked to NMDA receptors in most of the CNS synapses, but there are biased-positive allosteric modulators (PAMs) of mGlu5 receptors that enhance mGlu5 receptor function without recruiting NMDA receptors (4, 5). There are also dogmas in the mGlu field that have been recently challenged. For example, mGlu3 receptors are generally considered as presynaptic receptors that function to restrain neurotransmitter release. In contrast, recent evidence indicates that mGlu3 receptors are also present in postsynaptic elements, where they boost mGlu5 receptor signaling (6). mGlu3 and mGlu5 receptors synergize to induce long-term depression (LTD) in the mouse PFC, and mGlu3-saturating LTD requires the activation of mGlu5 receptors (6). This receptor-receptor interaction may be relevant to the pathophysiology and treatment of schizophrenia (see below).

mGlu receptors were discovered in the mid eighties (7, 8), but no mGlu receptor ligands are currently available for clinical use in spite of more than 30 years of extensive research. In our opinion, schizophrenia and Parkinson's disease are the two disorders in which the mGlu research will first translate into the clinic. In section mGlu2 receptors and schizophrenia: to be or no to be? we will comment on a series of clinical studies in which pomaglumetad, the prodrug of the mGlu2/3 receptor agonist, LY404039, has been tested in patients affected by schizophrenia. Although pomaglumetad is no longer under clinical development, these studies attracted more and more interest on mGlu receptors in schizophrenia. It is generally believed that the activity of LY404039 in preclinical models and in subgroups of patients affected by schizophrenia is mediated by the activation of mGlu2 receptors. Here we will challenge the ≪ mGlu2-centric ≫ hypothesis of schizophrenia suggesting that mGlu3 receptors might be at least as valuable as mGlu2 receptors as condadate drug targets for schizophrenia. mGlu2 and mGlu3 receptors show important differences in the expression and function in the tripartite synapsis (pre- and post-synaptic elements, and astrocytes), and modulate the activity of microglial cells in an opposite fashion. We will also discuss the rationale behind the development of mGlu5 receptor PAMs and the attractive possibility that mGlu3 receptor agonists or PAMs might produce disease modifying effects in schizophrenia. Finally, we will comment on the attractive possibility that activation of mGlu1 receptors represents a valuable strategy in the treatment of positive symptoms of schizophrenia, and we will conclude with the discussion of a new set of evidence suggesting that mGlu4 receptors are also candidate drug targets for schizophrenia.

METHODS

We searched for the following terms on Pubmed: metabotropic glutamate receptors and schizophrenia, metabotropic glutamate receptors and psychosis, metabotropic glutamate receptors and neurodevelopment, metabotropic glutamate receptors and CNS development, metabotropic glutamate receptors and network activity.

TARGETING mGlu5 AND mGlu3 RECEPTORS TO CORRECT NEURODEVELOPMENTAL ALTERATIONS ASSOCIATED WITH SCHIZOPHRENIA

A large body of evidence suggests that schizophrenia is a neurodevelopmental disorder in which cortical interneurons become dysfunctional as a result of genetic alterations or environmental challenges occurring in the perinatal period. There are at least four processes that characterize the development of cortical GABAergic interneurons: (i) the biochemical specification into different cell types, of which those containing parvalbumin (PV) or somatostatin (SSt)

are the most numerous; (ii) the correct matching between interneurons and pyramidal neurons (9); (iii) the GABA shift from excitatory into inhibitory driven by the expression of the potassium-chloride symport, KCC2 in mature neurons (10); and (iv) the formation of perineuronal nets (PNNs), which surround PV^+ interneurons at the offset of the critical temporal windows of developmental plasticity (11, 12). The molecular events that drive these processes are only partially elucidated. Schizophrenia is associated with a dysfunction of PV^+ (basket and chandelier) and other populations of interneurons, with a resulting defect in network oscillations underlying cognitive functions (13) (**Figure 1**). In addition, several lines of evidence suggest that formation of PNNs is altered in the PFC of patients affected by schizophrenia (14–16). What may link mGlu3 and mGlu5 receptors to these processes is the evidence that the expression of both receptors is high in the early postnatal development, and then progressively declines to reach adult levels after weaning (17). One of the first observations associated with the discovery of mGlu receptors was that mGlu receptor-mediated polyphosphoinositide (PI) hydrolysis (a biochemical process leading to intracellular Ca^{2+} mobilization and activation of protein kinase C) is dramatically high in the cerebral cortex and other brain regions during the early 7–9 days of postnatal life, and declines afterwards (8). This robust PI response is mediated by mGlu5 receptors, which are heavily expressed early after birth even in cells that lack mGlu5 receptors in the adult life, such as cerebellar Purkinje cells (18). An unexpected finding was that a large component of mGlu5-mediated PI hydrolysis in the developing PFC was lost in $mGlu3^{-/-}$ mice, and, therefore, required the endogenous activation of mGlu3 receptors (6). In contrast, mGlu5 receptor-mediated PI hydrolysis was intact in $mGlu2^{-/-}$ mice (6). Interestingly, expression of mGlu2 receptors in the cerebral cortex is low in the early postnatal life and increases afterwards (as opposed to expression of mGlu3 and mGlu5 receptors) (17).

Which (if any) of the neurodevelopmental processes taking place early after birth is/are driven by the combined activation of mGlu3 and mGlu5 receptors? This question is starting to be investigated. A reduction in the transcript and protein levels of PV, SSt, GAD-65, GAD-67, Reelin, and AMPA and NMDA receptor subunits has been reported in the PFC of adult $mGlu5^{-/-}$ mice (19). In $mGlu3^{-/-}$ mice we found changes in biochemical markers of cortical GABAergic interneurons during the first 4 weeks of postnatal development, which were not seen in

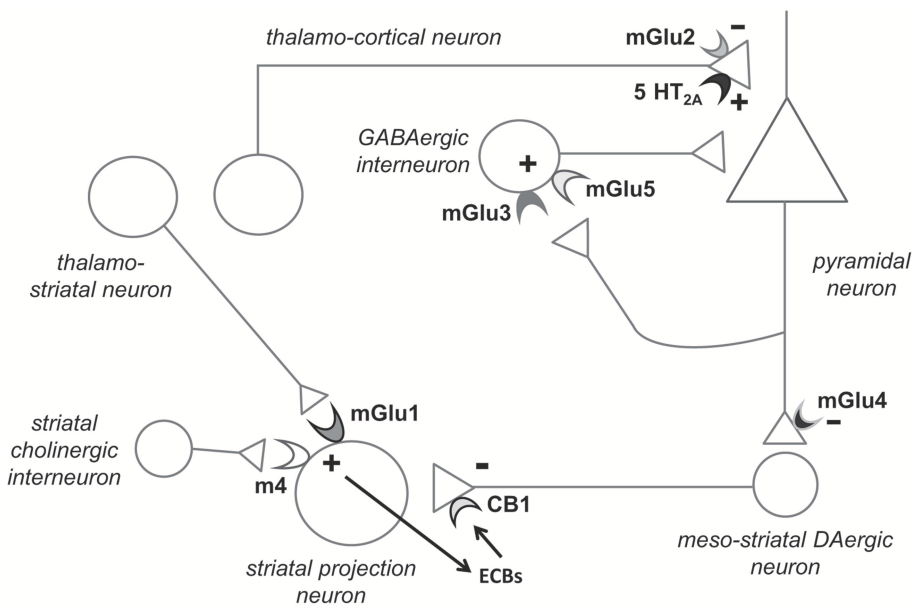

FIGURE 1 | Hypothetical model highlighting the potential role of individual mGlu receptor subtypes in the pathophysiology and treatment of schizophrenia. Presynaptic mGlu2 receptors negatively modulate 5-HT_{2A} receptor signaling, and their activation restrains glutamate release at synapses between thalamo-cortical neurons and cortical pyramidal neurons (see chapter 6). mGlu5 receptors are found in both pyramidal neurons and interneurons (shown here) and functionally interact with NMDA receptors. mGlu5 receptor PAMs are under development for the treatment of schizophrenia (see chapter 4). Recent evidence indicates that mGlu3 receptor activation boost mGlu5 receptor signaling, and, therefore, selective mGlu3 receptor agonists or PAMs should be tested in preclinical models predictive of antipsychotic activity (chapter 3). The functional cross-talk between mGlu3 and mGlu5 receptors has been shown in pyramidal neurons (6). We speculate that the two receptors also interact in GABAergic interneurons, but this remains to be demonstrated. mGlu1 receptors and type-4 muscarinic cholinergic receptors (M4) interact in stimulating the production of endocannabinoids (ECBs) in striatal projection neurons. ECBs activate presynaptic CB1 receptors, thereby reducing dopamine (DA) release from meso-striatal terminals. Hence, mGlu1 receptor PAMs might be effective in improving positive symptoms in schizophrenia (chapter 5). Finally, mGlu4 receptor agonists/PAMs are effective in animal models that are predictive of antipsychotic activity. mGlu4 receptors might act to reduce glutamate release from excitatory nerve endings of pyramidal neurons and through other mechanisms. Not shown here: activation of mGlu3 and mGlu5 receptors may restrain neuroinflammation by driving microglia toward an antinflammatory phenotype, and activation of mGlu3 receptors stimulates the production of neurotrophic factors in astrocytes (see chapters 3 and 4).

mGlu2$^{-/-}$ mice (20). These findings suggest that the functional partnership between mGlu3 and mGlu5 receptors may be instrumental for the biochemical and functional specialization of cortical GABAergic interneurons. An attractive hypothesis is that the two receptors may also serve to regulate the numeric balance between pyramidal neurons and GABAergic interneurons in the developing cerebral cortex. An elegant article by Prof. Oscar Marin and his Associates (9) demonstrates that the balanced network between excitatory and inhibitory cortical neurons becomes established through mechanisms of activity-dependent cell death and survival, with the decrease in the total number of pyramidal neurons preceding the decrease in the number of interneurons in the first few days of postnatal life. Surviving interneurons are those that are innervated by pyramidal cells because synaptic excitation enhances the activity of the phosphatidylinositol-3-kinase (PI3K) pathway by suppressing the expression of the PI3K negative regulator, phosphatase and tensin homolog PTEN (9). Both mGlu3 and mGlu5 receptors may signal through the activation of the PI3K pathway (3), and we are currently examining whether genetic and pharmacological manipulation of the two receptors alters the expression of total and phosphorylated PTEN in the developing cerebral cortex. Another interesting area of investigation is whether mGlu5 and mGlu3 receptors regulate KCC2 expression and GABA responses in pyramidal cells during postnatal development. Changes in cortical and cerebellar KCC2 levels were found in mice lacking mGlu3 and mGlu5 receptors, respectively (20, 21).

How all this can be relevant to the pathophysiology and experimental treatment of schizophrenia? Cell-specific deletion of mGlu5 receptors in PV^{+} neurons in mice results into a behavioral phenotype modeling schizophrenia, characterized by spontaneous hyperactivity, and defects in pre-pulse inhibition and long-term memory (22). A psychotic-like phenotype was also observed in mice lacking mGlu3 receptors (23, 24). Wang et al. (2) found that mGlu5 receptor signaling was largely reduced in the post-mortem DLPFC of individuals affected by schizophrenia, as shown by tyrosine hyperphosphorylation (leading to receptor desensitization), reductions in receptor coupling to $G_{q/11}$, Homer, and PI3K, and reduced receptor association with adaptor and scaffolding proteins, such as RGS4, norbin, Preso 1 and tamalin. Remarkably, the reduced signaling of mGlu5 receptors disrupted the physiological interplay between mGlu5 and NMDA receptors in the DLPFC of individuals affected by schizophrenia (2). Abnormalities in mGlu3 receptor dimerization have also been reported in the frontal cortex of individuals affected by schizophrenia (25). Polymorphic variants of GRM3 (the gene encoding for the mGlu3 receptor) have been consistently associated with schizophrenia (26–37), and genetic alterations of GRM5 have also been linked to schizophrenia (38).

We hypothesize that, in schizophrenia, a reduced expression and/or activity of mGlu3 and mGlu5 receptors may have a strong impact on the maturation of cortical GABAergic interneurons leading to a permanent alteration of network activity in the frontal cortex. If turned to be correct, this hypothesis raises the interesting possibility that a pharmacological intervention with mGlu3 or mGlu5 receptor PAMs early after birth may restore the correct developmental trajectory rescuing the pathological phenotype. This is a testable hypothesis in animal models, but translation to humans is an extremely difficult task for ethical and practical reasons. Regardless of any potential therapeutical application, defining the precise role of mGlu3 and mGlu5 receptors in neuronal development will give an answer to an intriguing question raised since the discovery of mGlu receptors: why mGlu receptor-mediated PI hydrolysis is so large in the early postnatal life (much larger than PI hydrolysis mediated by any other receptor coupled to $G_{q/11}$) and progressively declines afterwards?

Another potential link between mGlu5 receptor signaling and schizophrenia is suggested by the evidence that the endogenous D-amino acid, D-aspartate, has a great efficacy in stimulating PI hydrolysis in cortical or hippocampal slices prepared from 8-9 day-old rats, and its action is largely mediated by mGlu5 receptors (39). D-Aspartate levels are reduced in the dorsolateral prefrontal cortex of patients affected by schizophrenia as a result of an enhanced activity of the catabolic enzyme, D-aspartate oxidase (40, 41). In addition, the antipsychotic drug, olanzapine, enhances glutamate release in the prefrontal cortex by inhibiting D-aspartate catabolism (42). Thus, a defective D-aspartate/mGlu5 receptor axis might contribute to the pathophysiology of schizophrenia, and some antipsychotic drugs might reinforce mGlu5 receptor signaling by enhancing endogenous D-aspartate levels. Whether D-aspartate directly activates mGlu5 receptors, or receptor activation is secondary to the enhanced glutamate release induced by D-aspartate remains to be determined.

mGlu5 RECEPTOR PAMS AS NOVEL ANTIPSYCHOTIC AGENTS

The leading hypothesis of NMDA receptor hypofunction in schizophrenia stems from the strong psychotomimetic effects of slow NMDA receptor channel blockers, such as phenyclidine (PCP) and ketamine, the cognitive dysfunction and psychosis caused by NMDA receptor autoantibodies in patients affected by autoimmune encephalitis and the large body of evidence obtained in mice lacking NMDA receptors or treated with NMDA receptor antagonists. NMDA receptors are found in most CNS neurons; however, they are highly expressed and functional in cortical interneurons (43). Several drugs that activate NMDA receptors have been developed for the treatment of schizophrenia, but with limited success in clinical trials. One notable example is bitopertin, a drug that inhibits the high affinity uptake of the NMDA receptor co-agonist, glycine. After promising preclinical and phase-2 clinical studies, bitopertin failed in phase-3 trials (44). Although clinical data with bitopertin were disappointing, active work on NMDA receptor agonists continues. A functional cross-talk exists between mGlu5 and NMDA receptors. Activation of mGlu5 receptors facilitates NMDA receptor function (45–50), whereas activation of NMDA receptors amplifies mGlu5 receptor activity by restraining receptor desensitization (51). This and other observations laid the groundwork for the development of mGlu5 reptor PAMs for the treatment of schizophrenia. Jeff Conn, Carrie Jones,

Craig Lidsley and their Associates (Vanderbilt University) are actively involved in the design and development of highly selective and brain permeant mGlu5 receptor PAMs, and they are so kind to make their molecules available to the scientific community. PAMs differ from orthosteric agonists because they have no intrinsic efficacy but amplify the action of endogenous glutamate. Thus, PAMs are particularly advantageous from a therapeutical standpoint because their action is activity-dependent. Systemic treatment with mGlu5 PAMs corrects the pathological phenotype in pharmacological and genetic models that have predictive validity for antipsychotic treatment, including models of NMDA receptor hypofunction (52–58) (**Figure 1**). Excitotoxicity mediated by the enhanced NMDA receptor activity is a potential limtation to the use of mGlu5 receptor PAMs in the treatment of schizophrenia. Accordingly, high doses of mGlu5 receptor PAMs may cause seizures and neurotoxicity in rodents (4, 59, 60). An elegant way to overcome these limitations is the use of biased mGlu5 receptor PAMs that amplify receptor function without recruiting NMDA receptors (4, 5). One of these molecules, compound VU0409551, showed antipsychotic-like activity without activating NMDA receptors (5).

Biased mGlu5 PAMs might be particularly helpful in the treatment of patients affected by schizophrenia who are resistant to conventional antipsychotic medication. Our expectation is that these drugs may improve both positive and negative symptoms and exert pro-cognitive effects by restoring the balance between excitation and inhibition in the prefrontal cortex of patients affected by schizophrenia (58). While an early treatment with mGlu5 PAMs might correct the developmental abnormalities associated with schizophrenia (see above), the impact of a late treatment on disease progression cannot be predicted. A large body of evidence suggests that neuroinflammation has a key role in the progressive degeneration of the gray and white matter associated with schizophrenia (61–63). Interestingly, mGlu5 receptors are present in microglial cells, and their activation drives microglia toward an anti-inflammatory phenotype (64–69).

A potential anti-inflammatory effect of mGlu5 PAMs may slow disease progression in the absence of a direct induction of excitotoxic neuronal death. Thus, biased PAMs that enhance mGlu5 receptor activation without potentiating NMDA receptor currents cater the potential to behave as disease modifyiers in schizophrenia. This interesting hypothesis warrants further investigation in animal models of psychosis associated with neuroinflammation.

TARGETING mGlu1 RECEPTORS IN THE TREATMENT OF PSYCHOSIS

An elegant manuscript describes a novel form of receptor-receptor interaction involving M4 muscarinic acetylcholine receptors and mGlu1 receptors (70). This interaction might pave the way to novel therapeutic strategies in schizophrenia. M4 receptor activation inhibits striatal dopamine release by enhancing the production of endocannabinoids, which behave as retrograde messengers at dopaminergic nerve terminals. Interestingly, this mechanism requires the co-activation of mGlu1 receptors, which are coupled to $G_{q/11}$, and, therefore, have the *fisique du role* to enhance the production of endocannabinoids. M4 receptors, which are coupled to $G_{i/o}$, are also able to inhibit D1 receptor signaling, but this function is independent of mGlu1 receptors (70) (**Figure 1**). Inhibition of dopamine release mediated by mGlu1 receptors explains the antipsychotic-like activity of selective mGlu1 PAMs in rodents (70) and holds promise for the treatment of positive symptoms of schizophrenia. Mutations of GRM1 (the gene encoding for the mGlu1 receptor) that reduce mGlu1 receptor signaling have been associated with schizophrenia (71), and mice lacking mGlu1 receptors display a psychotic-like phenotype (72). This suggests that a defective mGlu1 receptor expression and/or activity might contribute to the striatal dopaminergic hyperactivity associated with schizophrenia, and that mGlu1 receptor PAMs may correct this defect. Of note, co-activation of M4 and mGlu1 receptors selectively inhibits dopamine release in the striatum without affecting dopaminergic transmission in other brain regions. This suggests that mGlu1 receptor PAMs may reduce dopamine release only *where is needed*, i.e., in the hyperactive meso-striatal system. This is advantageous with respect to most antisychotic agents, which block D2 receptors with no regional discrimination.

The interplay between mGlu1 and M4 receptors is one of the many examples of functional interactions between $G_{q/11}$-coupled group-I mGlu receptors and other $G_{i/o}$ coupled receptors. In the cerebellum, $GABA_B$ receptors cooperate with mGlu1 receptors at the synapses between parallel fibers and Purkinje cells (73). Andrzej Pilc and his Associates (Krakow University, Polland) have described a synergistic effect between mGlu5 and $GABA_B$ receptor PAMs in behavioral tests with pharmacological validity for the treatment of positive, negative and cognitive symptoms of schizophrenia (74).

mGlu2 RECEPTORS AND SCHIZOPHRENIA: TO BE OR NO TO BE?

The study of mGlu2 receptors as druggable targets in schizophrenia has been a leading theme of research in the whole mGlu receptor field in the last two decades. The evidence that compound LY354740 (a potent, selective, and brain permeant mGlu2/3 agonist) was able to reverse behavioral and neurochemical effects of PCP in rats (75) gave the impetus to the development of orthosteric mGlu2/3 receptor agonists or mGlu2 receptor PAMs as novel "non-monoaminergic" antipsychotic agents. mGlu2 receptors are presynaptic receptors that inhibit neurotransmitter release (3). It is believed that mGlu2 receptor activation produces antipsychotic effects by restraining the activity of 5-HT_{2A} receptors in the frontal cortex. Gerard Marek and his Associates were first to demonstrate that mGlu2 receptors inhibit electrophysiological responses mediated by 5-HT_{2A} receptors (76, 77). In the following years, Javier Gonzalez-Maeso and his Associates showed that mGlu2 receptors form a multimeric complex with 5-HT_{2A} receptors

and negatively modulate 5-HT_{2A} receptor signaling in response to serotonin-like hallucinogenic drugs (78–81). Expression of mGlu2 receptors in the mouse frontal cortex is under the control of 5-HT_{2A} receptors, and repeated administrations of second-generation antipsychotics, which block 5-HT_{2A} receptors, down-regulates the expression of mGlu2 receptors through an epigenetic mechanism mediated by reduced histone acetylation at the GRM2 gene promoter (82, 83). Interestingly, mGlu2 receptors are down-regulated in the frontal cortex of mice subjected to prenatal stress (84, 85), which models the epigenetic and behavioral modifications associated with schizophrenia (84, 86). Not surprisingly, both mGlu2/3 agonists and mGlu2 PAMs display robust antipsychotic-like activity in a variety of behavioral tests (87–90).

The clinical development of the mGlu2/3 agonist, LY404039 (under the form of the oral prodrug, pomaglumetad methionil) is a remarkable example of how translational research in schizophrenia is conditioned by a number of unpredictable variables. Pomaglumetad methionil was as efficacious as olanzapine in improving positive and negative symptoms of schizophrenia in the first phase 2 clinical trial (91) (**Figure 1**). Treatment with pomaglumetad methionil did not cause the typical adverse effects of antipsychotic drugs, such as extrapyramidal symptoms, increase in body weight, and hyperprolactinemia (91). These findings generated great enthusiasm, demonstrating for the first time the efficacy of a non-monoaminergic agent in the treatment of schizophrenia. Unfortunately, the antipsychotic activity of pomaglumetad methionil was not confirmed in subsequent phase 2 or 3 clinical trials (92–94), and this led to discontinuation of the development program of LY404039 in schizophrenia with negative consequences for the whole mGlu receptor field. However, a metanalysis of all clinical studies showed that pomeglumetad methionil displayed therapeutic efficacy in subgroups of patients who were early-in-disease and had never been treated with 5-HT_{2A} blocking agents (95). Research on the effect of LY404039 in humans is still ongoing, and both pomaglumetad methionil and LY2979165 (the oral prodrug of the selective mGlu2 receptor agonist, 2812223) were found to reduce ketamine-evoked blood oxygenation level dependent (BOLD) MRI signal in healthy subjects (96). It will be interesting to design clinical trials in which mGlu2/3 receptor agonists (or mGlu2 receptor PAMs) are tested in selected populations of patients affected by schizophrenia recruited on the basis of their genetic background, disease course, and history of previous medication. We are in favor of the development of mixed mGlu2/3 agonists or PAMs (rather than selective mGlu2 PAMs) if we consider the potential impact of all these drugs on disease progression. We have evidence that selective activation of mGlu2 receptors may produce neurotoxic effect (97–99) by enhancing the proinflammatory activity of microglia (100–102) or through other mechanisms. In contrast, activation of mGlu3 receptors is consistently neuroprotective through a mechanism of astrocyte-neuronal interaction mediated by the production of neurotrophic factors, such as transforming-growth factor-β1 or glial cell-derived neurotrophic factor (98, 103–105).

mGlu4 RECEPTORS AS NOVEL TARGETS FOR THE TREATMENT OF SCHIZOPHRENIA

The study of individual group-III mGlu receptor subtypes in schizophrenia is now facilitated by the availability of brain permeant subtype-selective agents that are suitable for *in vivo* studies. One of these drugs is compound LSP4-2022, which behaves as a preferential orthosteric agonist of mGlu4 receptors (106). Systemic treatment with LSP4-2022 has been shown to induce antipsychotic-like activity in a number of behavioral tests and to attenuate neurotransmitter release induced by the NMDA receptor antagonist, MK-801 (107, 108). Interestingly, mGlu4 receptors co-operate with other neurotransmitter receptors coupled to $G_{i/o}$ in improving psychotic-like symptoms in mice. Wozniak et al. (108) showed that the antipsychotic-like activity of LSP4-2022 in mice was prevented by pharmacological blockade of 5-HT_{1A} receptors, whereas sub-threshold doses of LSP4-2022 and the 5-HT_{1A} agonist, 8-hydroxy-dipropylaminotetraline (8-OH-DPAT), acted synergistically in producing antipsychotic- like effects. A similar synergism was shown between LSP4-2022 and drugs that activate $GABA_B$ receptors, although in this case the synergism could only be demonstrated in behavioral tests that model positive symptoms of schizophrenia (107).

A more recent study exended the analysis to the interaction between mGlu4 and M4 muscarinic receptors in an attempt to develop novel pharmacological strategies with good efficacy in improving positive, negative and cognitive symptoms, and good profile of safety and tolerability. A robust effect was seen by combining subactive doses of LSP4-2022 and the selective M4 muscarinic receptor PAM, VU152100, in behavioral tests that model positive, negative, and cognitive symptoms. This combination also reduced 5-HT_{2A}-mediated spontaneous excitatory postsynaptic currents in frontal cortical slices, indicating that mGlu4 and M4 receptors cooperate in reducing glutamate release (109). Remarkably, the association of subactive doses of drugs that activate mGlu4 and M4 receptors did not cause motor impairment in mice, suggesting that the functional cross-talk between mGlu4 and M4 receptors can be targeted by new safer antipsychotic agents (**Figure 1**).

Finally, the study of mGlu4 receptors provides a new potential link beween the kynurenine pathway and schizophrenia. The kynurenine pathway of tryptophan metabolism generates a series of neuroactive componds, including the mGlu4 receptor agonist, cinnabarinic acid (110). We have evidence that cinnabarinic acid inhibits behavioral and biochemical responses to MK-801 at very low doses (111). Cinnabarinic acid is synthesized in very low amounts in the normal brain, but its production increases considerably under conditions of neuroinflammation. A protective activity of endogenous cinnabarinic acid against neuroinflammation (112) and psychotic symptoms (113) might be lost in schizophrenia because of a lower activity of kynurenine monoxygenase (114–116), the enzyme that converts kynurenine into 3-hydroxykynurenine giving rise to all metabolites that lie downstream of 3-hydroxykynurenine. This is certainly a field of great interest that warrants further investigation.

CONCLUSIONS

Subtype-selective mGlu receptor ligands offer the opportunity of a precision medicine based pharmacological approach to target multiple domains of the psychopathological spectrum of schizophrenia. For example, patients with mutations of GRM1 or GRM3 genes might benefit from a treatment with mGlu1 and mGlu3 receptor PAMs, respectively, whereas patients who are early in disease or had never been treated with second-generation antipsychotics might respond to mGlu2/3 receptor agonists. The increasing knowledge of the role played by mGlu3 and mGlu5 receptors in the developmental trajectory of GABAergic interneurons might pave the way to an early use of mGlu3 or mGlu5 receptor PAMs as disease modifying agents, being aware that treatment of schizophrenia in the preclinical phase awaits the discovery of predictive biomarkers endowed with high specificity and selectivity. Finally, some mGlu receptor ligands might have a positive impact on neuroinflammation by influencing microglial function or through other mechanisms. There are no reasons to be skeptical and continue to develop new mGlu receptor ligands for the treatment of schizophrenia with good hope of success.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Vita A, De Peri L, Deste G, Barlati S, Sacchetti E. The effect of antipsychotic treatment on cortical gray matter changes in schizophrenia: does the class matter? A meta-analysis and meta-regression of longitudinal magnetic resonance imaging studies. *Biol Psychiatry* (2015) 78:403–12. doi: 10.1016/j.biopsych.2015.02.008
2. Wang HY, MacDonald ML, Borgmann-Winter KE, Banerjee A, Sleiman P, Tom A, et al. mGluR5 hypofunction is integral to glutamatergic dysregulation in schizophrenia. *Mol Psychiatry* (2018). doi: 10.1038/s41380-018-0234-y. [Epub ahead of print].
3. Nicoletti F, Bockaert J, Collingridge GL, Conn PJ, Ferraguti F, Schoepp DD, et al. Metabotropic glutamate receptors: from the workbench to the bedside. *Neuropharmacology* (2011) 60:1017–41. doi: 10.1016/j.neuropharm.2010.10.022
4. Rook JM, Noetzel MJ, Pouliot WA, Bridges TM, Vinson PN, Cho HP, et al. Unique signaling profiles of positive allosteric modulators of metabotropic glutamate receptor subtype 5 determine differences in *in vivo* activity. *Biol Psychiatry* (2013) 73:501–9. doi: 10.1016/j.biopsych.2012.09.012
5. Rook JM, Xiang Z, Lv X, Ghoshal A, Dickerson JW, Bridges TM, et al. Biased mGlu5-positive allosteric modulators provide *in vivo* efficacy without potentiating mGlu5 modulation of NMDAR currents. *Neuron* (2015) 86:1029–40. doi: 10.1016/j.neuron.2015.03.063
6. Di Menna L, Joffe ME, Iacovelli L, Orlando R, Lindsley CW, Mairesse J, et al. Functional partnership between mGlu3 and mGlu5 metabotropic glutamate receptors in the central nervous system. *Neuropharmacology* (2018) 128:301–13. doi: 10.1016/j.neuropharm.2017.10.026
7. Sladeczek F, Pin JP, Récasens M, Bockaert J, Weiss S. Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature* (1985) 317:717–9. doi: 10.1038/317717a0
8. Nicoletti F, Iadarola MJ, Wroblewski JT, Costa E. Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: developmental changes and interaction with alpha 1-adrenoceptors. *Proc Natl Acad Sci USA*. (1986) 83:1931–5. doi: 10.1073/pnas.83.6.1931
9. Wong FK, Bercsenyi K, Sreenivasan V, Portalés A, Fernández-Otero M, Marín O. Pyramidal cell regulation of interneuron survival sculpts cortical networks. *Nature* (2018) 557:668–73. doi: 10.1038/s41586-018-0139-6
10. Ben-Ari Y. The GABA excitatory/inhibitory developmental sequence: a personal journey. *Neuroscience* (2014) 279:187–219. doi: 10.1016/j.neuroscience.2014.08.001
11. Pizzorusso T, Medini P, Berardi N, Chierzi S, Fawcett JW, Maffei L. Reactivation of ocular dominance plasticity in the adult visual cortex. *Science* (2002) 298:1248–51. doi: 10.1126/science.1072699
12. Ueno H, Suemitsu S, Okamoto M, Matsumoto Y, Ishihara T. Parvalbumin neurons and perineuronal nets in the mouse prefrontal cortex. *Neuroscience* (2017) 343:115–27. doi: 10.1016/j.neuroscience.2016.11.035
13. Gonzalez-Burgos G, Cho RY, Lewis DA. Alterations in cortical network oscillations and parvalbumin neurons in schizophrenia. *Biol Psychiatry* (2015) 77:1031–40. doi: 10.1016/j.biopsych.2015.03.010
14. Pantazopoulos H, Woo TU, Lim MP, Lange N, Berretta S. 2010. Extracellular matrix-glial abnormalities in the amygdala and entorhinal cortex of subjects diagnosed with schizophrenia. *Arch Gen Psychiatry* (2010) 67:155–66. doi: 10.1001/archgenpsychiatry.2009.196
15. Berretta S. Extracellular matrix abnormalities in schizophrenia. *Neuropharmacology* (2012). 62:1584–97. doi: 10.1016/j.neuropharm.2011.08.010
16. Berretta S, Pantazopoulos H, Markota M, Brown C, Batzianouli ET. Losing the sugar coating: potential impact of perineuronal net abnormalities on interneurons in schizophrenia. *Schizophr Res* (2015) 167:18–27. doi: 10.1016/j.schres.2014.12.040
17. Catania MV, Landwehrmeyer GB, Testa CM, Standaert DG, Penney JB Jr, Young AB. Metabotropic glutamate receptors are differentially regulated during development. *Neuroscience* (1994) 61:481–95. doi: 10.1016/0306-4522(94)90428-6
18. Notartomaso S, Nakao H, Mascio G, Scarselli P, Cannella M, Zappulla C, et al. mGlu1 receptors monopolize the synaptic control of cerebellar Purkinje cells by epigenetically down-regulating mGlu5 receptors. *Sci Rep*. (2018) 8:13361. doi: 10.1038/s41598-018-31369-7
19. Luoni A, Gass P, Brambilla P, Ruggeri M, Riva MA, Inta D. Altered expression of schizophrenia-related genes in mice lacking mGlu5 receptors. *Eur Arch Psychiatry Clin Neurosci*. (2018) 268:77–87. doi: 10.1007/s00406-016-0728-z
20. Battaglia G, Imbriglio T, Verhaeghe R, Martinello K, Del Percio C, Pascarelli MT, et al. Genetic Deletion of mGlu3 Receptors Causes Developmental Abnormalities in GABAergic Neurons and Cortical Neural Synchronization in Mice: A Multi-Modal and -Scale Approach. Program No. 122.05. In: *2018 Neuroscience Meeting Planner*. San Diego, CA: Society for Neuroscience (2018).
21. Notartomaso S, Mascio G, Scarselli P, Martinello K, Fucile S, Gradini R, et al. Expression of the $K(^{+})/Cl(^{-})$ cotransporter, KCC2, in cerebellar Purkinje cells is regulated by group-I metabotropic glutamate receptors. *Neuropharmacology* (2017) 115:51–59. doi: 10.1016/j.neuropharm.2016.07.032
22. Barnes SA, Pinto-Duarte A, Kappe A, Zembrzycki A, Metzler A, Mukamel EA, et al. Disruption of mGluR5 in parvalbumin-positive interneurons induces core features of neurodevelopmental disorders. *Mol Psychiatry* (2015) 20:1161–72. doi: 10.1038/mp.2015.113
23. Fujioka R, Nii T, Iwaki A, Shibata A, Ito I, Kitaichi K, et al. Comprehensive behavioral study of mGluR3 knockout mice: implication in schizophrenia related endophenotypes. *Mol Brain* (2014) 23:31. doi: 10.1186/1756-6606-7-31
24. Lainiola M, Procaccini C, Linden AM. mGluR3 knockout mice show a working memory defect and an enhanced response to MK-801 in the T- and Y-maze cognitive tests. *Behav Brain Res*. (2014) 266:94–103. doi: 10.1016/j.bbr.2014.03.008
25. Corti C, Crepaldi L, Mion S, Roth AL, Xuereb JH, Ferraguti F. Altered dimerization of metabotropic glutamate receptor 3 in schizophrenia. *Biol Psychiatry* (2007) 62:747–55. doi: 10.1016/j.biopsych.2006.12.005

26. Fujii Y, Shibata H, Kikuta R, Makino C, Tani A, Hirata N, et al. Positive associations of polymorphisms in the metabotropic glutamate receptor type 3 gene (GRM3) with schizophrenia. *Psychiatr Genet*. (2003) 13:71–76. doi: 10.1097/01.ypg.0000056682.82896.b0
27. Fijal BA, Kinon BJ, Kapur S, Stauffer VL, Conley RR, Jamal HH, et al. Candidate-gene association analysis of response to risperidone in African-American and white patients with schizophrenia. *Pharmacogenomics J*. (2009) 9:311–18. doi: 10.1038/tpj.2009.24
28. Egan MF, Straub RE, Goldberg TE, Yakub I, Callicott JH, Hariri AR, et al. Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia. *Proc Natl Acad Sci USA*. (2004) 101:12604–609. doi: 10.1073/pnas.0405077101
29. Chen Q, He G, Chen Q, Wu S, Xu Y, Feng G, et al. A case-control study of the relationship between the metabotropic glutamate receptor 3 gene and schizophrenia in the Chinese population. *Schizophr Res*. (2005) 73:21–6. doi: 10.1016/j.schres.2004.07.002
30. Norton N, Williams HJ, Dwyer S, Ivanov D, Preece AC, Gerrish A, et al. No evidence for association between polymorphisms in GRM3 and schizophrenia. *BMC Psychiatry* (2005) 13:5–23. doi: 10.1186/1471-244X-5-23
31. Bishop JR, Ellingrod VL, Moline J, Miller D. Association between the polymorphic GRM3 gene and negative symptom improvement during olanzapine treatment. *Schizophr Res*. (2005) 77:253–60. doi: 10.1016/j.schres.2005.04.001
32. Bishop JR, Wang K, Moline J, Ellingrod VL. Association analysis of the metabotropic glutamate receptor type 3 gene (GRM3) with schizophrenia. *Psychiatr Genet* (2007) 17:358. doi: 10.1097/YPG.0b013e3281ac231e
33. Bishop JR, Miller del D, Ellingrod VL, Holman T. Association between type-three metabotropic glutamate receptor gene (GRM3) variants and symptom presentation in treatment refractory schizophrenia. *Hum Psychopharmacol.* (2011) 26:28–34. doi: 10.1002/hup.1163
34. Schwab SG, Plummer C, Albus M, Borrmann-Hassenbach M, Lerer B, Trixler M, et al. DNA sequence variants in the metabotropic glutamate receptor 3 and risk to schizophrenia: an association study. *Psychiatr Genet* (2008) 18:25–30. doi: 10.1097/YPG.0b013e3282ef48d9
35. Sartorius LJ, Weinberger DR, Hyde TM, Harrison PJ, Kleinman JE, Lipska BK. Expression of a GRM3 splice variant is increased in the dorsolateral prefrontal cortex of individuals carrying a schizophrenia risk SNP. *Neuropsychopharmacology* (2008) 33:2626–34. doi: 10.1038/sj.npp.1301669
36. Maj C, Minelli A, Giacopuzzi E, Sacchetti E, Gennarelli M. The role of metabotropic glutamate receptor genes in schizophrenia. *Curr Neuropharmacol.* (2016) 14:540–50. doi: 10.2174/1570159X13666150514232745
37. Saini SM, Mancuso SG, Mostaid MS, Liu C, Pantelis C, Everall IP, et al. Meta-analysis supports GWAS-implicated link between GRM3 and schizophrenia risk. *Transl Psychiatry* (2017) 7:e1196. doi: 10.1038/tp.2017.172
38. Devon RS, Anderson S, Teague PW, Muir WJ, Murray V, Pelosi AJ, et al. The genomic organisation of the metabotropic glutamate receptor subtype 5 gene, and its association with schizophrenia. *Mol Psychiatry* (2001) 6:311–14. doi: 10.1038/sj.mp.4000848
39. Molinaro G, Pietracupa S, Di Menna L, Pescatori L, Usiello A, Battaglia G, et al. D-aspartate activates mGlu receptors coupled to polyphosphoinositide hydrolysis in neonate rat brain slices. *Neurosci Lett*. (2010) 478:128–30. doi: 10.1016/j.neulet.2010.04.077
40. Errico F, Napolitano F, Squillace M, Vitucci D, Blasi G, de Bartolomeis A, et al. Decreased levels of D-aspartate and NMDA in the prefrontal cortex and striatum of patients with schizophrenia. *J Psychiatr Res*. (2013) 47:1432–7. doi: 10.1016/j.jpsychires.2013.06.013
41. Nuzzo T, Sacchi S, Errico F, Keller S, Palumbo O, Florio E, et al. Decreased free d-aspartate levels are linked to enhanced d-aspartate oxidase activity in the dorsolateral prefrontal cortex of schizophrenia patients. *NPJ Schizophr*. (2017) 3:16. doi: 10.1038/s41537-017-0015-7
42. Sacchi S, Novellis V, Paolone G, Nuzzo T, Iannotta M, Belardo C, et al. Olanzapine, but not clozapine, increases glutamate release in the prefrontal cortex of freely moving mice by inhibiting D-aspartate oxidase activity. *Sci Rep*. (2017) 7:46288. doi: 10.1038/srep46288
43. Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* (2012) 37:4–15. doi: 10.1038/npp.2011.181
44. Bugarski-Kirola D, Blaettler T, Arango C, Fleischhacker WW, Garibaldi G, Wang A, et al. Bitopertin in negative symptoms of schizophrenia-results from the phase III FlashLyte and DayLyte studies. *Biol Psychiatry* (2017) 82:8–16. doi: 10.1016/j.biopsych.2016.11.014
45. Doherty AJ, Palmer MJ, Henley JM, Collingridge GL, Jane DE. (RS)-2-Chloro-5- hydroxyphenylglycine (CHPG) activates mGlu5, but no mGlu1, receptors expressed in CHO cells and potentiates NMDA responses in the hippocampus. *Neuropharmacology* (1997) 36:265–67. doi: 10.1016/S0028-3908(97)00001-4
46. Ugolini A, Corsi M, Bordi F. Potentiation of NMDA and AMPA responses by the specific mGluR5 agonist CHPG in spinal cord motoneurons. *Neuropharmacology* (1999) 38:1569–76. doi: 10.1016/S0028-3908(99)00095-7
47. Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ. Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *J Neurosci*. (2000) 20:7871–879. doi: 10.1523/JNEUROSCI.20-21-07871.2000
48. Attucci S, Carlà V, Mannaioni G, Moroni F. Activation of type 5 metabotropic glutamate receptors enhances NMDA responses in mice cortical wedges. *Br J Pharmacol* (2001) 132:799–806. doi: 10.1038/sj.bjp.0703904
49. Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ. Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. *J Neurosci.* (2001) 21:5925–34. doi: 10.1523/JNEUROSCI.21-16-05925.2001
50. Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, et al. Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-d-aspartate responses in medium spiny striatal neurons. *Neuroscience* (2001) 106:579–87. doi: 10.1016/S0306-4522(01)00297-4
51. Alagarsamy S, Saugstad J, Warren L, Mansuy IM, Gereau RW 4th, Conn PJ. NMDA-induced potentiation of mGluR5 is mediated by activation of protein phosphatase 2B/calcineurin. *Neuropharmacology* (2005) 49 (Suppl. 1):135–45. doi: 10.1016/j.neuropharm.2005.05.005
52. Kinney GG, Burno M, Campbell UC, Hernandez LM, Rodriguez D, Bristow LJ, et al. Metabotropic glutamate subtype 5 receptors modulate locomotor activity and sensorimotor gating in rodents. *J Pharmacol Exp Ther*. (2003) 306:116–23. doi: 10.1124/jpet.103.048702
53. Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, et al. A novel selective positive allosteric modulator of metabotropic glutamate receptor subtype 5 has *in vivo* activity and antipsychotic-like effects in rat behavioral models. *J Pharmacol Exp Ther*. (2005) 313:199–206. doi: 10.1124/jpet.104.079244
54. Lecourtier L, Homayoun H, Tamagnan G, Moghaddam B. Positive allosteric modulation of metabotropic glutamate 5 (mGlu5) receptors reverses N-Methyl- D-aspartate antagonist-induced alteration of neuronal firing in prefrontal cortex. *Biol Psychiatry* (2007) 62:739–46. doi: 10.1016/j.biopsych.2006.12.003
55. Liu F, Grauer S, Kelley C, Navarra R, Graf R, Zhang G, et al. ADX47273 [S-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone]: a novel metabotropic glutamate receptor 5-selective positive allosteric modulator with preclinical antipsychotic-like and procognitive activities. *J Pharmacol Exp Ther*. (2008) 327:827–39. doi: 10.1124/jpet.108.136580
56. Gastambide F, Gilmour G, Robbins TW, Tricklebank MD. The $mGlu_5$ positive allosteric modulator LSN2463359 differentially modulates motor, instrumental and cognitive effects of NMDA receptor antagonists in the rat. *Neuropharmacology* (2013) 64:240–47. doi: 10.1016/j.neuropharm.2012.07.039
57. Balu DT, Li Y, Takagi S, Presti KT, Ramikie TS, Rook JM, et al. An mGlu5-Positive allosteric modulator rescues the neuroplasticity deficits in a genetic model of NMDA receptor hypofunction in schizophrenia. *Neuropsychopharmacology* (2016) 41:2052–61. doi: 10.1038/npp.2016.2
58. Stansley BJ, Conn PJ. The therapeutic potential of metabotropic glutamate receptor modulation for schizophrenia. *Curr Opin Pharmacol.* (2018) 38:31–36. doi: 10.1016/j.coph.2018.02.003
59. Parmentier-Batteur S, Hutson PH, Menzel K, Uslaner JM, Mattson BA, O'Brien JA, et al. Mechanism based neurotoxicity of mGlu5

positive allosteric modulators–development challenges for a promising novel antipsychotic target. *Neuropharmacology* (2014) 82:161–73. doi: 10.1016/j.neuropharm.2012.12.003

60. Conde-Ceide S, Alcázar J, Alonso de Diego SA, López S, Martín-Martín ML, Martínez-Viturro CM, et al. Preliminary investigation of 6,7-dihydropyrazolo[1,5-a]pyrazin-4-one derivatives as a novel series of mGlu5 receptor positive allosteric modulators with efficacy in preclinical models of schizophrenia. *Bioorg Med Chem Lett*. (2016) 26:429–34. doi: 10.1016/j.bmcl.2015.11.098
61. Müller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. *Front Neurosci*. (2015) 9:372. doi: 10.3389/fnins.2015.00372
62. Trépanier MO, Hopperton KE, Mizrahi R, Mechawar N, Bazinet RP. Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. *Mol Psychiatry* (2016) 21:1009–26. doi: 10.1038/mp.2016.90
63. Miller BJ, Goldsmith DR. Towards an immunophenotype of schizophrenia: progress, potential mechanisms, and future directions. *Neuropsychopharmacology* (2017) 42:299–317. doi: 10.1038/npp.2016.211
64. Byrnes KR, Stoica B, Loane DJ, Riccio A, Davis MI, Faden AI. Metabotropic glutamate receptor 5 activation inhibits microglial associated inflammation and neurotoxicity. *Glia* (2009) 57:550–60. doi: 10.1002/glia.20783
65. Loane DJ, Stoica BA, Pajoohesh-Ganji A, Byrnes KR, Faden AI. Activation of metabotropic glutamate receptor 5 modulates microglial reactivity and neurotoxicity by inhibiting NADPH oxidase. *J Biol Chem*. (2009) 284:15629–39. doi: 10.1074/jbc.M806139200
66. Piers TM, Heales SJ, Pocock JM. Positive allosteric modulation of metabotropic glutamate receptor 5 down-regulates fibrinogen-activated microglia providing neuronal protection. *Neurosci Lett*. (2011) 505:140–5. doi: 10.1016/j.neulet.2011.10.007
67. Xue F, Stoica BA, Hanscom M, Kabadi SV, Faden AI. Positive allosteric modulators (PAMs) of metabotropic glutamate receptor 5 (mGluR5) attenuate microglial activation. *CNS Neurol Disord Drug Targets* (2014) 13:558–66. doi: 10.2174/18715273113126660199
68. Qiu JL, Zhu WL, Lu YJ, Bai ZF, Liu ZG, Zhao P, et al. The selective mGluR5 agonist CHPG attenuates SO2-induced oxidative stress and inflammation through TSG-6/NF-κB pathway in BV2 microglial cells. *Neurochem Int*. (2015) 85–86:46–52. doi: 10.1016/j.neuint.2015.04.007
69. Hanak TJ, Libbey JE, Doty DJ, Sim JT, DePaula-Silva AB, Fujinami RS. Positive modulation of mGluR5 attenuates seizures and reduces TNF-α(+) macrophages and microglia in the brain in a murine model of virus-induced temporal lobe epilepsy. *Exp Neurol*. (2018) 311:194–204. doi: 10.1016/j.expneurol.2018.10.006
70. Yohn SE, Foster DJ, Covey DP, Moehle MS, Galbraith J, Garcia-Barrantes PM, et al. Activation of the mGlu(1) metabotropic glutamate receptor has antipsychotic-like effects and is required for efficacy of M(4) muscarinic receptor allosteric modulators. *Mol Psychiatry* (2018). doi: 10.1038/s41380-018-0206-2. [Epub ahead of print].
71. Cho HP, Garcia-Barrantes PM, Brogan JT, Hopkins CR, Niswender CM, Rodriguez AL, et al. Chemical modulation of mutant mGlu1 receptors derived from deleterious GRM1 mutations found in schizophrenics. *ACS Chem Biol*. (2014) 17:2334–46. doi: 10.1021/cb500560h
72. Brody SA, Conquet F, Geyer MA. (2003) Disruption of prepulse inhibition in mice lacking mGluR1. *Eur J Neurosci*. (2003)18:3361–66. doi: 10.1111/j.1460-9568.2003.03073.x
73. Tabata T, Araishi K, Hashimoto K, Hashimotodani Y, van der Putten H, Bettler B, et al. Ca^{2+} activity at GABAB receptors constitutively promotes metabotropic glutamate signaling in the absence of GABA. *Proc Natl Acad Sci USA*. (2004) 101:16952–7. doi: 10.1073/pnas.0405387101
74. Wieronska JM, Kłeczek N, Wozniak M, Gruca P, Łason-Tyburkiewicz M, Papp M, et al. $mGlu_5$-$GABA_B$ interplay in animal models of positive, negative and cognitive symptoms of schizophrenia. *Neurochem Int*. (2015) 88:97–109. doi: 10.1016/j.neuint.2015.03.010
75. Moghaddam B, Adams BW. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* (1998) 281:1349–52. doi: 10.1126/science.281.5381.1349
76. Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. *Brain Res Brain Res Rev*. (2000) 31:302–12. doi: 10.1016/S0165-0173(99)00046-6
77. Marek GJ, Wright RA, Schoepp DD, Monn JA, Aghajanian GK. 2000. Physiological antagonism between 5-hydroxytryptamine(2A) and group II metabotropic glutamate receptors in prefrontal cortex. *J Pharmacol Exp Ther*. (2000) 292:76–87.
78. Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, López-Giménez JF, et al. Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* (2008) 452:93–7. doi: 10.1038/nature06612
79. Gonzalez-Maeso J. Allosteric signaling through an mGlu2 and 5-HT_{2A} heteromeric receptor complex and its potential contribution to schizophrenia. *Sci Signal* (2016) 9:ra5. doi: 10.1126/scisignal.aab0467
80. Fribourg M, Moreno JL, Holloway T, Provasi D, Baki L, Mahajan R, et al. Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. *Cell* (2011) 147:1011–23. doi: 10.1016/j.cell.2011.09.055
81. Delille HK, Mezler M, Marek GJ. The two faces of the pharmacological interaction of $mGlu_2$ and 5-HT_{2A} – relevance of receptor heterocomplexes and interaction through functional brain pathways. *Neuropharmacology* (2013) 70:296e305. doi: 10.1016/j.neuropharm.2013.02.005
82. Kurita M, Holloway T, García-Bea A, Kozlenkov A, Friedman AK, Moreno JL, et al. HDAC2 regulates atypical antipsychotic responses through the modulation of mGlu2 promoter activity. *Nat Neurosci*. (2012) 15:1245–54. doi: 10.1038/nn.3181
83. de la Fuente Revenga M, Ibi D, Cuddy T, Toneatti R, Kurita M, Ijaz MK, et al. Chronic clozapine treatment restrains via HDAC2 the performance of mGlu2 receptor agonism in a rodent model of antipsychotic activity. *Neuropsychopharmacology* (2018) 44:443–54. doi: 10.1038/s41386-018-0143-4
84. Matrisciano F, Tueting P, Maccari S, Nicoletti F, Guidotti A. Pharmacological activation of group-II metabotropic glutamate receptors corrects a schizophrenia-like phenotype induced by prenatal stress in mice. *Neuro Psychopharmacol*. (2012) 37:929–38. doi: 10.1038/npp.2011.274
85. Holloway T, Moreno JL, Umali A, Rayannavar V, Hodes GE, Russo SJ, et al. Prenatal stress induces schizophrenia-like alterations of serotonin 2A and metabotropic glutamate 2 receptors in the adult offspring: role of maternal immune system. *J Neurosci*. (2013) 33:1088–98. doi: 10.1523/JNEUROSCI.2331-12.2013
86. Dong Z, Sun X, Pan C, Lu T, Han Y, Wang L, et al. Association of DISC1, BDNF, and COMT polymorphisms with exploratory eye movement of schizophrenia in a Chinese Han population. *Psychiatr Genet* (2016) 26:258–265 doi: 10.1097/YPG.0000000000000138
87. Field JR, Walker AG, Conn PJ. Targeting glutamate synapses in schizophrenia. *Trends Mol Med*. (2011) 17:689–98. doi: 10.1016/j.molmed.2011.08.004
88. Fell MJ, McKinzie DL, Monn JA, Svensson KA. Group II metabotropic glutamate receptor agonists and positive allosteric modulators as novel treatments for schizophrenia. *Neuropharmacology* (2012) 62:1473–83. doi: 10.1016/j.neuropharm.2011.06.007
89. Nikiforuk A, Popik P, Drescher KU, van Gaalen M, Relo AL, Mezler M, et al. Effects of a positive allosteric modulator of group II metabotropic glutamate receptors, LY487379, on cognitive flexibility and impulsive-like responding in rats. *J Pharmacol Exp Ther*. (2010) 335:665–73. doi: 10.1124/jpet.110.170506
90. Hiyoshi T, Marumo T, Hikichi H, Tomishima Y, Urabe H, Tamita T, et al. Neurophysiologic and antipsychotic profiles of TASP0433864, a novel positive allosteric modulator of metabotropic glutamate 2 receptor. *J Pharmacol Exp Ther*. (2014) 351:642–53. doi: 10.1124/jpet.114.218651
91. Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. *Nat Med*. (2007) 13:1102–7. doi: 10.1038/nm1632
92. Kinon BJ, Potts AJ, Watson SB. Placebo response in clinical trials with schizophrenia patients. *Curr Opin Psychiatry* (2011) 24:107–13. doi: 10.1097/YCO.0b013e32834381b0

93. Stauffer VL, Millen BA, Andersen S, Kinon BJ, Lagrandeur L, Lindenmayer JP, et al. Pomaglumetad methionil: no significant difference as an adjunctive treatment for patients with prominent negative symptoms of schizophrenia compared to placebo. *Schizophr Res*. (2013) 150:434–41. doi: 10.1016/j.schres.2013.08.020
94. Downing AM, Kinon BJ, Millen BA, Zhang L, Liu L, Morozova MA, et al. A double-blind, placebo-controlled comparator study of LY21400 23 monohydrate in patients with schizophrenia. *BMC Psychiatry* (2014) 14:351. doi: 10.1186/s12888-014-0351-3
95. Kinon BJ, Millen BA, Zhang L, McKinzie DL. Exploratory analysis for a targeted patient population responsive to the metabotropic glutamate 2/3 receptor agonist pomaglumetad methionil in schizophrenia. *Biol Psychiatry* (2015) 78:754–62. doi: 10.1016/j.biopsych.2015.03.016
96. Mehta MA, Schmechtig A, Kotoula V, McColm J, Jackson K, Brittain C, et al. Group II metabotropic glutamate receptor agonist prodrugs LY2979165 and LY2140023 attenuate the functional imaging response to ketamine in healthy subjects. *Psychopharmacology* (2018) 235:1875–86. doi: 10.1007/s00213-018-4877-9
97. Corti C, Battaglia G, Molinaro G, Riozzi B, Pittaluga A, Corsi M, et al. The use of knock-out mice unravels distinct roles for mGlu2 and mGlu3 metabotropic glutamate receptors in mechanisms of neurodegeneration/neuroprotection. *J Neurosci*. (2007) 27:8297–308. doi: 10.1523/JNEUROSCI.1889-07.2007
98. Caraci F, Molinaro G, Battaglia G, Giuffrida ML, Riozzi B, Traficante A, et al. Targeting group II metabotropic glutamate (mGlu) receptors for the treatment of psychosis associated with Alzheimer's disease: selective activation of mGlu2 receptors amplifies beta-amyloid toxicity in cultured neurons, whereas dual activation of mGlu2 and mGlu3 receptors is neuroprotective. *Mol Pharmacol*. (2011) 79:618–26. doi: 10.1124/mol.110.067488
99. Motolese, M., Mastroiacovo, F., Cannella, M., Bucci, D., Gaglione, A., Riozzi, B., et al. Targeting type-2 metabotropic glutamate receptors to protect vulnerable hippocampal neurons against ischemic damage. *Mol Brain* (2015) 8:66. doi: 10.1186/s13041-015-0158-2
100. Taylor DL, Diemel LT, Cuzner ML, Pocock JM. Activation of group II metabotropic glutamate receptors underlies microglial reactivity and neurotoxicity following stimulation with chromogranin A, a peptide up-regulated in Alzheimer's disease. *J Neurochem*. (2002) 82:1179–91. doi: 10.1046/j.1471-4159.2002.01062.x
101. Taylor DL, Jones F, Kubota ES, Pocock JM. Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha- induced neurotoxicity in concert with microglial-derived Fas ligand. *J Neurosci*. (2005) 25:2952–64. doi: 10.1523/JNEUROSCI.4456-04.2005
102. Kaushal V, Schlichter LC. Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. *J Neurosci*. (2008) 28:2221–30. doi: 10.1523/JNEUROSCI.5643-07.2008
103. Bruno V, Battaglia G, Casabona G, Copani A, Caciagli F, Nicoletti F. (1998) Neuroprotection by glial metabotropic glutamate receptors is mediated by transforming growth factor-beta. *J Neurosci*. (1998) 18:9594–600. doi: 10.1523/JNEUROSCI.18-23-09594.1998
104. Battaglia G, Molinaro G, Riozzi B, Storto M, Busceti CL, Spinsanti P, et al. Activation of mGlu3 receptors stimulates the production of GDNF in striatal neurons. *PLoS ONE* (2009) 4:e6591. doi: 10.1371/journal.pone.0006591
105. Battaglia G, Riozzi B, Bucci D, Di Menna L, Molinaro G, Pallottino S, et al. Activation of mGlu3 metabotropic glutamate receptors enhances GDNF and GLT-1 formation in the spinal cord and rescues motor neurons in the SOD-1 mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis*. (2015) 74:126–36. doi: 10.1016/j.nbd.2014.11.012
106. Goudet C, Vilar B, Courtiol T, Deltheil T, Bessiron T, Brabet I, et al. A novel selective metabotropic glutamate receptor 4 agonist reveals new possibilities for developing subtype selective ligands with therapeutic potential. *FASEB J*. (2012) 26:1682–93. doi: 10.1096/fj.11-195941
107. Wozniak M, Acher F, Marciniak M, Lason-Tyburkiewicz M, Gruca P, Papp M, et al. Involvement of GABAB receptor signaling in antipsychotic-like action of the novel orthosteric agonist of the mGlu4 receptor, LSP4-2022. *Curr Neuropharmacol*. (2016) 14:413–26. doi: 10.2174/1570159X13666150516000630
108. Wozniak M, Gołembiowska K, Noworyta-Sokołowska K, Acher F, Cieślik P, Kusek M, et al. Neurochemical and behavioral studies on the 5-HT(1A)-dependent antipsychotic action of the mGlu(4) receptor agonist LSP4-2022. *Neuropharmacology* (2017) 115:149–65. doi: 10.1016/j.neuropharm.2016.06.025
109. Cieślik P, Wozniak M, Rook JM, Tantawy MN, Conn PJ, Acher F, et al. Mutual activation of glutamatergic $mGlu_{(4)}$ and muscarinic $M_(4)$ receptors reverses schizophrenia-related changes in rodents. *Psychopharmacology* (2018) 235:2897–2913. doi: 10.1007/s00213-018-4980-y
110. Fazio F, Lionetto L, Molinaro G, Bertrand HO, Acher F, Ngomba RT, et al. Cinnabarinic acid, an endogenous metabolite of the kynurenine pathway, activates type 4 metabotropic glutamate receptors. *Mol Pharmacol*. (2012) 81:643–56. doi: 10.1124/mol.111.074765
111. Fazio F, Ulivieri M, Volpi C, Gargaro M, Fallarino F. Targeting metabotropic glutamate receptors for the treatment of neuroinflammation. Curr Opin Pharmacol (2018) 38:16–23. doi: 10.1016/j.coph.2018.01.010
112. Fazio F, Zappulla C, Notartomaso S, Busceti C, Bessede A, Scarselli P, et al. Cinnabarinic acid, an endogenous agonist of type-4 metabotropic glutamate receptor, suppresses experimental autoimmune encephalomyelitis in mice. *Neuropharmacology* (2014) 81:237–43. doi: 10.1016/j.neuropharm.2014.02.011
113. Fazio F, Ulivieri M, Wieronska JM, Cieślik P, Mascio G, Traficante A, et al. Antipsychotic-like Activity of the Kynurenine Metabolite, Cinnabarinic Acid, in Mice. Program No. 122.07. In: *2018 Neuroscience Meeting Planner*. San Diego, CA: Society for Neuroscience (2018).
114. Aoyama N, Takahashi N, Saito S, Maeno N, Ishihara R, Ji X, et al. Association study between kynurenine 3-monooxygenase gene and schizophrenia in the Japanese population. *Genes Brain Behav* (2006) 5:364–68. doi: 10.1111/j.1601-183X.2006.00231.x
115. Wonodi I, Stine OC, Sathyasaikumar KV, Roberts RC, Mitchell BD, Hong LE, et al. Downregulated kynurenine 3-monooxygenase gene expression and enzyme activity in schizophrenia and genetic association with schizophrenia endophenotypes. *Arch Gen Psychiatry* (2011) 68:665–74. doi: 10.1001/archgenpsychiatry.2011.71
116. Sathyasaikumar KV, Stachowski EK, Wonodi I, Roberts RC, Rassoulpour A, McMahon RP, et al. Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. *Schizophr Bull*. (2011) 37:1147–56. doi: 10.1093/schbul/sbq112

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 21 June 2019
doi: 10.3389/fpsyt.2019.00440

Autism Associated With Anti-NMDAR Encephalitis: Glutamate-Related Therapy

*Ruu-Fen Tzang[1,2], Chuan-Hsin Chang[3], Yue-Cune Chang[4] and Hsien-Yuan Lane[5,6,7]**

[1] Department of Psychiatry, Mackay Memorial Hospital, Taipei, Taiwan, [2] Mackay Medicine, Nursing and Management College, Taipei, Taiwan, [3] Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan, [4] Department of Mathematics, Tamkang University, Taipei, Taiwan, [5] Department of Psychiatry, China Medical University Hospital, Taichung, Taiwan, [6] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, [7] Department of Psychology, College of Medical and Health Sciences, Asia University, Taichung, Taiwan

The purpose of this review is to correlate autism with autoimmune dysfunction in the absence of an explanation for the etiology of autism spectrum disorder. The anti-N-methyl-D-aspartate receptor (anti-NMDAR) autoantibody is a typical synaptic protein that can bind to synaptic NMDA glutamate receptors, leading to dysfunctional glutamate neurotransmission in the brain that manifests as psychiatric symptoms (psychosis, hallucinations, and personality changes). Detection of autoantibodies, cytokines, decreased lymphocytes, serum immunoglobulin level imbalance, T-cell mediated immune profile, maternal infection history, and children's infection history can all be vital biological markers of autoimmune autism. Diagnosing autoimmune encephalitis sooner can increase the effectiveness of curative treatments—such as immune therapy or immune modulatory therapy—that may prevent the long-term consequence of being misdiagnosed with autism spectrum disorder. Glutamate therapy primarily normalizes glutamate neurotransmission and can be a new add-on intervention alongside antipsychotics for treating autoimmune autism.

Keywords: autoimmune autism, glutamate-related therapy, anti-N-methyl-D-aspartate receptor encephalitis, immune therapy, dysfunctional glutamate neurotransmission

OPEN ACCESS

Edited by:
Ming D. Li,
Zhejiang University, China

Reviewed by:
Alessandro Usiello,
Second University of Naples, Italy
Ming D. Li,
Zhejiang University, China
Zhongli Yang,
Zhejiang University, China

***Correspondence:**
Hsien-Yuan Lane
hylane@gmail.com

Specialty section:
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *07 November 2018*
Accepted: *03 June 2019*
Published: *21 June 2019*

Citation:
Tzang R-F, Chang C-H, Chang Y-C and Lane H-Y (2019) Autism Associated With Anti-NMDAR Encephalitis: Glutamate-Related Therapy. Front. Psychiatry 10:440. doi: 10.3389/fpsyt.2019.00440

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by symptoms of impaired communication, stereotyped behavior, difficulty with social interaction, and certain repetitive or unusual behaviors. The current prevalence of ASD is estimated to be 1.5% or higher in developed countries (1); however, the etiology of autism remains largely unexplained. At the beginning of the 1980s, researchers began to apply the "immune hypothesis of schizophrenia" to explain immune-dysfunction–induced neuroinflammation as a cause for the symptoms of schizophrenia (2). Since autism has some overlapping features with schizophrenia, for example both have disturbed cognitive and social function, and there are neurobiological (brain volumes) and genetic (e.g., involvement of the same genes or chromosomal locations) domains both in autism and schizophrenia (3). Therefore, researchers also apply "neuroinflammation hypothesis of ASD" to regard autism as a disorder of autoimmune dysfunction (4, 5) or as autoimmune autism.

At least 69% of individuals with a diagnosis of ASD have been known to have neuroinflammation or encephalitis (6). Specifically, the so-called "anti-brain autoantibody" may damage fetal or children's brain cells, eventually leading to children falling into an autistic or regressive state.

Such brain-reactive antibodies causing autistic symptoms may elucidate the exploration of autism's etiology and suggest practical anti-inflammatory management protocols for children with ASD (7). Thus, this review introduces the potential pathogenicity of autism, which could explain why autoimmune dysfunction leads to autistic symptoms (indicated herein as "autoimmune autism").

Furthermore, we emphasize that psychiatrists should more quickly recognize autoimmune encephalitis—anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis in children and adolescent patients and treat them as soon as possible, because timely immune therapy or glutamate therapy is beneficial for children with autism. In addition, through a literature review, we review four hypothetical pathways that correlate autism with dysfunctional autoimmunity.

DYSFUNCTIONAL AUTOIMMUNITY-ORIGIN FROM MOTHER

On the basis of evidence that some of children with autism have mothers with rheumatoid arthritis, celiac disease, or a family history of type 1 diabetes (8), the symptoms of autism can be explained using the following exposure model. The fetus is exposed to prenatal antibodies from the dysfunctional maternal familial autoimmune system. The maternal pathogenic IgG antibodies or "induced maternal autoantibody-related ASD antibodies" cross the placenta and interact with the receptors in the fetal brain, eventually resulting in disruption of the brain cells (9–11).

An infection in the mother or child can trigger more brain damage in the fetus. If a mother with possible maternal familial autoimmune dysfunction develops an infection during pregnancy, this infection can trigger the autoimmune dysfunction to become severe, and maternal prenatal antibodies will then start attacking the brain of the fetus, leading to symptoms of autism (8); this is called "maternal-infection–triggered immune dysfunction." Children may develop the social, functional, and behavioral deficits observed in ASD owing to impaired neurodevelopment triggered by infection (5). Therefore, history of maternal immune dysfunctional disease or maternal infection is vital for diagnosing autoimmune autism.

DYSFUNCTIONAL AUTOIMMUNITY-ORIGIN FROM CHILD

Laing et al. (1989) demonstrated that when rabbits were inoculated with influenza A virus, the induced antibodies cross-reacted with a brain-specific protein found in the hippocampus, cortex, and cerebellum of the rabbits and caused brain cell damage. Various viral infections or immunizations during a child's growth period—such as encephalitis associated with potassium channel complex antibody, NMDAR encephalitis, and Hashimoto's encephalitis or vaccination induced anti-NMDAR encephalitis (12, 13)—all may cause several types of autoimmune encephalitis (5).

The anti-NMDAR autoantibody is a typical synaptic protein found in the nerve cells of teratomas. This autoantibody can bind with synaptic NMDA glutamate receptors in the brain, thereby causing dysfunctional glutamate neurotransmission and leading to psychiatric symptoms, such as psychosis, hallucinations, and personality changes (14). Recently, anti-NMDAR encephalitis has been frequently noticed after viral infection rather than originating from teratomas. The virally induced anti-NMDAR autoantibody may also attack the NMDA receptors of brain cells, causing hypofunction of glutamate neurotransmission in children with autism.

Such anti-NMDAR encephalitis is a type of reversible neuron dysfunction caused by autoimmune dysfunctional autoantibodies against NMDAR (15). Several cases of anti-NMDAR encephalitis have indeed been reported in patients with autism (16–18). Children with anti-NMDAR encephalitis symptoms that mimicked autistic regression recovered after timely immune therapy (19–21).

Approximately 30%–70% of autistic patients have circulating anti-brain autoantibodies (4). The autoantibodies in autistic patients—including anticardiolipin, β2-Glycoprotein 1, and antiphosphoserine antibodies (21); anti-double-stranded DNA antibodies (22); and antinucleosome-specific antibodies (23)—all act as anti-brain autoantibodies and play roles in disrupting neurotransmission by attacking specific neurotransmitter receptors. More specific autoantibody detection tools should be developed clinically to help autistic patients receive an accurate diagnosis instead of being misdiagnosed with autism.

AUTOIMMUNE AUTISM AFTER INTERFACE THEORIES

Not all children develop autoimmune dysfunction after a viral infection. Through the "Immune-Mediated Two-Hit Model for Psychosis" theory, it can be understood that only the brains of a specific group of children will be attacked. These children possibly have mothers with activated monocytes or microglia caused by maternal stress and inflammation paradigms during a prior pregnancy. When inflammation or some viral infection hits the memorized immune system or activating microglia of children, these children develop severe autoimmune abnormalities, and the second hit blows their neuronal circuitry (24). Here, prior maternal stress and inflammation induce a memorized immune system or activate microglia in children's brains, leading to the development of autistic symptoms in children during infections that occur in the early neurodevelopmental period.

AUTOIMMUNE AUTISM—CYTOKINE LEVEL

Cytokines [including chemokines, interferons, interleukins (ILs), lymphokines, and tumor necrosis factor (TNF)] are small proteins used in cell signaling. Cytokine deregulation occurs after overactivation of the immune system because of infection, injury, or inflammation (4). Elevated serum levels of IL-6 and IL-17A (25), increased serum IL-6 level (26), and increased expression of the inflammatory molecules IL-1β, IL-6, IL-17, and TNF (27) have been observed in children with autism.

Proinflammatory cytokines promote inflammation and have also been found to be highly increased in the peripheral blood

of patients with an ASD diagnosis in comparison with controls (28, 29). Furthermore, the proinflammatory cytokines TNF-alpha, IL-6, granulocyte-macrophage colony-stimulating factor, Th1 cytokine (interferon-gamma), and chemokine (IL-8) are increased in the brains of individuals with ASD (30).

Most crucially, an increased level of one type of cytokine (IL-6) may alter the tryptophan/kynurenine pathway, which is closely related to glutamatergic neurotransmission (31). Assessing the aforementioned cytokine markers would allow researchers to diagnose autoimmune encephalitis accurately instead of misdiagnosing patients with autism. For children with autoimmune ASD symptom, the role of cytokines in ASD symptoms is that an autistic child may have autoimmune encephalitis induced glutamate hypofunction in his brain. Therefore, glutamate therapy could be used as the effective therapeutic strategy in restoring glutamate receptor's function (32) and cytokine alterations. However, no study is available on using cytokine as the biomarkers of immune function to assess the effect of glutamate therapy in either ASD or schizophrenia. That will be an interesting study in future.

AUTOIMMUNE AUTISM—T-CELL-MEDIATED AUTOIMMUNITY

In addition to an imbalance of cytokine levels in the serum or cerebrospinal fluid, signs of T-cell activation are reliable indicators of autoimmune dysfunction in autism. T-cell-mediated autoimmunity participates in the functions of physiological defense, maintenance, and brain repair. Autoimmune T-cell deficiency or T-cell imbalance alters the activation profile of T-cells (33)—the innate proinflammatory response—with increased T-cell activation or a skewed display (34). Moreover, it significantly increases the T helper cell 1 and 2 (Th1/Th2) ratio (30). All these actions are associated with autoimmune autism.

In summary, in *ex vivo* studies, immune cells including abnormal or skewed T helper cells (Th1 and Th2), cytokine profiles, decreased lymphocytes, decreased T-cell mitogen response, and an imbalance of serum immunoglobulin levels (35) have all been linked with autoimmune encephalitis and autistic symptoms.

AUTOIMMUNE AUTISM—NUCLEAR FACTOR KAPPA-LIGHT-CHAIN-ENHANCER OF ACTIVATED B-CELLS

Nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) is a protein found in almost all cell types. This protein mediates the regulation of cellular immune responses by promoting the expression of inflammatory cytokines and chemokines as well as by establishing a feedback mechanism that can produce chronic or excessive inflammation. Approximately 45% of a subgroup of children with autism have low natural killer (NK) cytotoxic cell activity (36). NF-κB is more aberrantly expressed in the orbitofrontal cortex of autistic patients than in controls. Specifically, the NF-κB of resident immune cells in brain regions are part of a molecular cascade indicating a more severe inflammation, which is associated with the behavioral and clinical symptoms of those with an ASD diagnosis (37).

AUTOIMMUNE AUTISM—IMMUNE-RELATED GENETIC POLYMORPHISM

Autism has been associated with autoimmune dysfunction and with immune-based genes including human leukocyte antigen (HLA)-DRB1 and complement C4 alleles. Such genes show aberrant immune activity during vulnerable and critical periods of neurodevelopment, participating in the generation of the neurological dysfunction characteristic of ASD (35). Higher expression of T-cell activation markers (HLA-DR, CD26) was noticed during a study of immune phenotyping of peripheral blood mononuclear cells in young autistic children but not in controls (38).

Additionally, patients with autism were discovered to have a significantly higher frequency of *HLA-DRB1*11* allele than controls (39). This joint analysis of genotype and DNA methylation broadly demonstrates the potential of both brain and blood-based DNA methylation for insights into ASD and psychiatric phenotypes (40). The 16p11.2 mutations altered kynurenine pathway metabolism leading to abnormal glutamatergic activity in autism and may be the pathogenesis of ASD (31). Ghaleiha et al. suggested to use Memantine as an adjunctive treatment to restore NMDAR-dependent functionality before (41). Moreover, Memantine had a function targeting glutamate neurotransmission and already found to be the potential new and safe adjunctive treatment in children with ASD (42, 43).

AUTOIMMUNE AUTISM—DIAGNOSIS

Social cognitive impairment in children with autism originates from dysfunction in dopaminergic, serotonergic, noradrenergic, and glutamatergic neurotransmission in the brain after dysfunctional autoimmunity. Consequently, patients who develop autoimmune autism early in life may be misdiagnosed if their anti-NMDAR encephalitis or potential autoimmune-related disease remains unrecognized (44). Autoimmune dysfunctional autism requires immune therapy; therefore, earlier detection is essential to prevent a misdiagnosis of autism. Detection of autoantibody, cytokines, decreased lymphocytes, imbalance of serum immunoglobulin levels, and T-cell-mediated immune profile in addition to maternal infection history or children's infection history can all be employed as biological markers of autoimmune autism.

AUTOIMMUNE AUTISM—TREATMENT

Autoimmune dysfunctional autism requires immune therapy, which involves first-line immune therapy with pulse therapy in addition to intravenous immunoglobulin and plasmapheresis. Second-line immune therapy comprises rituximab or cyclophosphamide. In several cases, favorable treatment effects were reported after immune therapy if the child was noticed to have NMDAR-Ab in the serum and cerebrospinal fluid (16, 19–20, 21, 34, 45, 46). Recently, ASD drug development has focused on correcting synaptic dysfunctions; abnormalities in central oxytocin, vasopressin, and serotonin neurotransmissions, as well as neuroinflammation targets for new strategies to treat the core symptoms of ASD (47).

TREATMENT: SECOND-LINE IMMUNE THERAPY

Up to half of all patients treated for anti-NMDAR encephalitis reported poor treatment response and the failure of first-line immunotherapy (46). Among these patients with inadequate treatment response, approximately 65% showed substantial improvement after well-tolerated second-line immunotherapy (46, 48). Second-line therapy, most commonly rituximab and/or cyclophosphamide, is often required in patients without tumors and those with a delayed diagnosis (49). Rituximab directed against the CD20 antigen on the surface of B-lymphocytes leads to decreased maturation of B-cells into antibody-secreting cells and is a favorable immunotherapy option in anti-NMDAR encephalitis (50). Second-line immunotherapy using rituximab has been reported to have improved the long-term outcome in a 16-year-old female patient with autoimmune NMDAR encephalitis and autistic traits diagnosed earlier in childhood (20, 21). Therefore, this study suggests using second-line immunotherapy as soon as possible to help patients with autoimmune autism who have had a poor response to treatment after first-line immune therapy of pulse therapy, plasmapheresis, or immunoglobulins.

AUTOIMMUNE AUTISM—GLUTAMATE-RELATED THERAPY

For patients with autoimmune autism who have responded poorly to immune therapy, have difficulty starting immune therapy, or are presently using antipsychotic treatment, immune-modulating therapy is the treatment of choice. Brain cells need glutamate (glutamic acid) to regulate the central nervous system. Accumulated neuroimaging, family, genetic and animal studies have demonstrated that glutamate (glutamic acid) can improve mood disturbance and executive function. In anti-NMDAR encephalitis, dysfunctional postsynaptic glutamatergic transmission in synapses leads to enhanced release of γ-Aminobutyric acid and hypofunction of glutamate secretion, thus the NMDAR agonist, e.g., D-cycloserine, sarcosine, and GLYX-13 (rapastinel) can modulate glutamatergic transmission (51). D-Cycloserine (DCS), a partial glycine B agonist at the NMDA receptor site, has been shown to improve sociability in patient with autism earlier (52) by normalizing glutamate neurotransmission. In recent years, D-cycloserine was also reported to be effective in improving stereotyped symptoms (53) and social reciprocity (54) in older adolescents and young adults with ASD.

Sodium benzoate, a D-amino acid oxidase inhibitor, was reported to be effective in children with communication difficulty with ASDs (55). Considering the challenge of earlier recognition of autoimmune autism, and the exorbitant cost of immune therapy in countries with poor medical knowledge of autoimmune autism, immune-modulating therapy or glutamate therapy can be a new add-on intervention alongside the current use of antipsychotics in treating autoimmune autism.

CONCLUSION

Earlier diagnosis of autoimmune encephalitis increases the potential of curative treatments by enabling provision of timely immune therapy or immune modulatory therapy, which can prevent long-term consequences, such as being misdiagnosed with autism (16). An immunophenotyped patient with symptoms of autism may need to obtain a diagnosis of autoimmune encephalitis to avoid being misdiagnosed with ASD. Additionally, in children with the underlying syndrome presentation of childhood disintegrative disorder, early-onset schizophrenia, or late-onset autism (56); in children with first episode of psychosis (20); and especially when autistic symptoms follow a febrile illness (44), autoimmune autism including diagnosis of anti-NMDAR encephalitis or other autoimmune dysfunctional encephalitis should be considered as a possible organic cause.

AUTHOR CONTRIBUTIONS

R-FT, C-HC, and H-YL produced the idea to this review. R-FT, C-HC, Y-CC, and H-YL did the literature review. R-FT and C-HC made the draft of this paper. H-YL critically revised this manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

Ministry of Science and Technology, Taiwan (MOST 107-2314-B-039-039); China Medical University, Taiwan (CMU107-BC-4); and China Medical University Hospital, Taiwan (DMR-108-093).

REFERENCES

1. Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, et al. The changing epidemiology of autism spectrum disorders. *Annu Rev Public Health* (2017) 38:81–102. doi: 10.1146/annurev-publhealth-031816-044318
2. Fudenberg HH, Whitten HD, Merler E, Farmati O. Is schizophrenia an immunologic receptor disorder? *Med Hypotheses* (1983) 12(1):85–93. doi: 10.1016/0306-9877(83)90037-3
3. Stone WS, Iguchi L. Do apparent overlaps between schizophrenia and autistic spectrum disorders reflect superficial similarities or etiological commonalities? *N Am J Med Sci (Boston)* (2011) 4(3):124–33. doi: 10.7156/v4i3p124
4. Ashwood P, Van de Water J. Is autism an autoimmune disease? *Autoimmun Rev* (2004) 3(7–8):557–62. doi: 10.1016/j.autrev.2004.07.036
5. Edmiston E, Ashwood P, Van de Water J. Autoimmunity, autoantibodies, and autism spectrum disorder. *Biol Psychiatry* (2017) 81(5):383–90. doi: 10.1016/j.biopsych.2016.08.031
6. Kern JK, Geier DA, Sykes LK, Geier MR. Relevance of neuroinflammation and encephalitis in autism. *Front Cell Neurosci* (2015) 9:519. doi: 10.3389/fncel.2015.00519
7. Brimberg L, Sadiq A, Gregersen PK, Diamond B. Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. *Mol Psychiatry* (2013) 18(11):1171–7. doi: 10.1038/mp.2013.101

8. Atladottir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, et al. Association of family history of autoimmune diseases and autism spectrum disorders. *Pediatrics* (2009) 124(2):687–94. doi: 10.1542/peds.2008-2445
9. Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, et al. Serologic evidence of prenatal influenza in the etiology of schizophrenia. *Arch Gen Psychiatry* (2004) 61(8):774–80. doi: 10.1001/archpsyc.61.8.774
10. Buka SL, Cannon TD, Torrey EF, Yolken RH. Maternal exposure to herpes simplex virus and risk of psychosis among adult offspring. *Biol Psychiatry* (2008) 63(8):809–15. doi: 10.1016/j.biopsych.2007.09.022
11. Klyushnik TP, Turkova IL, Danilovskaya EV, Kozlova IA, Bashina VM, Simashkova NV, et al. Correlation between levels of autoantibodies to nerve growth factor and the clinical features of schizophrenia in children. *Neurosci Behav Physiol* (2000) 30(2):119–21. doi: 10.1007/BF02463148
12. Wang H. Anti-NMDA receptor encephalitis and vaccination. *Int J Mol Sci* (2017) 18(1):193. doi: 10.3390/ijms18010193
13. Hofmann C, Baur MO, Schroten H. Anti-NMDA receptor encephalitis after TdaP-IPV booster vaccination: cause or coincidence? *J Neurol* (2011) 258(3):500–1. doi: 10.1007/s00415-010-5757-3
14. Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* (2007) 61(1):25–36. doi: 10.1002/ana.21050
15. Kayser MS, Titulaer MJ, Gresa-Arribas N, Dalmau J. Frequency and characteristics of isolated psychiatric episodes in anti-N-methyl-d-aspartate receptor encephalitis. *JAMA Neurol* (2013) 70(9):1133–9. doi: 10.1001/jamaneurol.2013.3216
16. Kiani R, Lawden M, Eames P, Critchley P, Bhaumik S, Odedra S, et al. Anti-NMDA-receptor encephalitis presenting with catatonia and neuroleptic malignant syndrome in patients with intellectual disability and autism. *BJPsych Bull* (2015) 39(1):32–5. doi: 10.1192/pb.bp.112.041954
17. Kurita D, Wakuda T, Takagai S, Takahashi Y, Iwata Y, Suzuki K, et al. Deterioration of clinical features of a patient with autism spectrum disorder after anti-N-methyl-D-aspartate receptor encephalitis. *Psychiatry Clin Neurosci* (2015) 69(8):507. doi: 10.1111/pcn.12323
18. Gonzalez-Toro MC, Jadraque-Rodriguez R, Sempere-Perez A, Martinez-Pastor P, Jover-Cerda J, Gomez-Gosalvez F. Anti-NMDA receptor encephalitis: two paediatric cases. *Rev Neurol* (2013) 57(11):504–8. doi: 10.33588/rn.5711.2013272
19. Hacohen Y, Wright S, Gadian J, Vincent A, Lim M, Wassmer E, et al. N-methyl-d-aspartate (NMDA) receptor antibodies encephalitis mimicking an autistic regression. *Dev Med Child Neurol* (2016) 58(10):1092–4. doi: 10.1111/dmcn.13169
20. Cleland N, Lieblich S, Schalling M, Rahm C. A 16-year-old girl with anti-NMDA-receptor encephalitis and family history of psychotic disorders. *Acta Neuropsychiatr* (2015) 27(6):375–9. doi: 10.1017/neu.2015.32
21. Careaga M, Hansen RL, Hertz-Piccotto I, Van de Water J, Ashwood P. Increased anti-phospholipid antibodies in autism spectrum disorders. *Mediators Inflamm* (2013) 2013:935608. doi: 10.1155/2013/935608
22. Mostafa GA, El-Sherif DF, Al-Ayadhi LY. Systemic auto-antibodies in children with autism. *J Neuroimmunol* (2014) 272(1–2):94–8. doi: 10.1016/j.jneuroim.2014.04.011
23. AL-A LY, Mostafa GA. Serum antinucleosome-specific antibody as a marker of autoimmunity in children with autism. *J Neuroinflammation* (2014) 11:69. doi: 10.1186/1742-2094-11-69
24. Bergink V, Gibney SM, Drexhage HA. Autoimmunity, inflammation, and psychosis: a search for peripheral markers. *Biol Psychiatry* (2014) 75(4):324–31. doi: 10.1016/j.biopsych.2013.09.037
25. Basheer S, Venkataswamy MM, Christopher R, Van Amelsvoort T, Srinath S, Girimaji SC, et al. Immune aberrations in children with autism spectrum disorder: a case-control study from a tertiary care neuropsychiatric hospital in India. *Psychoneuroendocrinology* (2018) 94:162–7. doi: 10.1016/j.psyneuen.2018.05.002
26. Tsilioni I, Taliou A, Francis K, Theoharides TC. Children with autism spectrum disorders, who improved with a luteolin-containing dietary formulation, show reduced serum levels of TNF and IL-6. *Transl Psychiatry* (2015) 5:e647. doi: 10.1038/tp.2015.142
27. Theoharides TC, Tsilioni I, Patel AB, Doyle R. Atopic diseases and inflammation of the brain in the pathogenesis of autism spectrum disorders. *Transl Psychiatry* (2016) 6(6):e844. doi: 10.1038/tp.2016.77
28. Zimmerman AW, Jyonouchi H, Comi AM, Connors SL, Milstien S, Varsou A, et al. Cerebrospinal fluid and serum markers of inflammation in autism. *Pediatr Neurol* (2005) 33(3):195–201. doi: 10.1016/j.pediatrneurol.2005.03.014
29. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, et al. Elevated cytokine levels in children with autism spectrum disorder. *J Neuroimmunol* (2006) 172(1–2):198–205. doi: 10.1016/j.jneuroim.2005.11.007
30. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, et al. Elevated immune response in the brain of autistic patients. *J Neuroimmunol* (2009) 207(1–2):111–6. doi: 10.1016/j.jneuroim.2008.12.002
31. Lim CK, Essa MM, de Paula Martins R, Lovejoy DB, Bilgin AA, Waly MI, et al. Altered kynurenine pathway metabolism in autism: implication for immune-induced glutamatergic activity. *Autism Res* (2016) 9(6):621–31. doi: 10.1002/aur.1565
32. Rojas DC. The role of glutamate and its receptors in autism and the use of glutamate receptor antagonists in treatment. *J Neural Transm (Vienna)* (2014) 121(8):891–905. doi: 10.1007/s00702-014-1216-0
33. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Altered T cell responses in children with autism. *Brain Behav Immun* (2011) 25(5):840–9. doi: 10.1016/j.bbi.2010.09.002
34. Careaga M, Rogers S, Hansen RL, Amaral DG, Van de Water J, Ashwood P. Immune endophenotypes in children with autism spectrum disorder. *Biol Psychiatry* (2017) 81(5):434–41. doi: 10.1016/j.biopsych.2015.08.036
35. Ashwood P, Wills S, Van de Water J. The immune response in autism: a new frontier for autism research. *J Leukoc Biol* (2006) 80(1):1–15. doi: 10.1189/jlb.1205707
36. Vojdani A, Mumper E, Granpeesheh D, Mielke L, Traver D, Bock K, et al. Low natural killer cell cytotoxic activity in autism: the role of glutathione, IL-2 and IL-15. *J Neuroimmunol* (2008) 205(1–2):148–54. doi: 10.1016/j.jneuroim.2008.09.005
37. Young AM, Campbell E, Lynch S, Suckling J, Powis SJ. Aberrant NF-kappaB expression in autism spectrum condition: a mechanism for neuroinflammation. *Front Psychiatry* (2011) 2:27. doi: 10.3389/fpsyt.2011.00027
38. Ashwood P, Corbett BA, Kantor A, Schulman H, Van de Water J, Amaral DG. In search of cellular immunophenotypes in the blood of children with autism. *PLoS One* (2011) 6(5):e19299. doi: 10.1371/journal.pone.0019299
39. Mostafa GA, Shehab AA, Al-Ayadhi LY. The link between some alleles on human leukocyte antigen system and autism in children. *J Neuroimmunol* (2013) 255(1–2):70–4. doi: 10.1016/j.jneuroim.2012.10.002
40. Andrews SV, Ellis SE, Bakulski KM, Sheppard B, Croen LA, Hertz-Picciotto I, et al. Cross-tissue integration of genetic and epigenetic data offers insight into autism spectrum disorder. *Nat Commun* (2017) 8(1):1011. doi: 10.1038/s41467-017-00868-y
41. Ghaleiha A, Asadabadi M, Mohammadi MR, Shahei M, Tabrizi M, Hajiaghaee R, et al. Memantine as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. *Int J Neuropsychopharmacol* (2013) 16(4):783–9. doi: 10.1017/S1461145712000880
42. Hage A, Banaschewski T, Buitelaar JK, Dijkhuizen RM, Franke B, Lythgoe DJ, et al. Glutamatergic medication in the treatment of obsessive compulsive disorder (OCD) and autism spectrum disorder (ASD) - study protocol for a randomised controlled trial. *Trials* (2016) 17(1):141. doi: 10.1186/s13063-016-1266-8
43. Aman MG, Findling RL, Hardan AY, Hendren RL, Melmed RD, Kehinde-Nelson O, et al. Safety and efficacy of memantine in children with autism: randomized, placebo-controlled study and open-label extension. *J Child Adolesc Psychopharmacol* (2017) 27(5):403–12. doi: 10.1089/cap.2015.0146
44. Scott O, Richer L, Forbes K, Sonnenberg L, Currie A, Eliyashevska M, et al. Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis: an unusual cause of autistic regression in a toddler. *J Child Neurol* (2014) 29(5):691–4. doi: 10.1177/0883073813501875
45. Kanako M. [Legislative problems concerning medical and welfare systems]. *Hokenfu Zasshi* (1974) 30(4):267–71.
46. Titulaer MJ, McCracken L, Gabilondo I, Armangue T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-term outcome in patients

with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol* (2013) 12(2):157–65. doi: 10.1016/S1474-4422(12)70310-1

47. Lacivita E, Perrone R, Margari L, Leopoldo M. Targets for drug therapy for autism spectrum disorder: challenges and future directions. *J Med Chem* (2017) 60(22):9114–41. doi: 10.1021/acs.jmedchem.7b00965
48. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol* (2011) 10(1):63–74. doi: 10.1016/S1474-4422(10)70253-2
49. Salvucci A, Devine IM, Hammond D, Sheth RD. Pediatric anti-NMDA (N-methyl D-aspartate) receptor encephalitis. *Pediatr Neurol* (2014) 50(5):507–10. doi: 10.1016/j.pediatrneurol.2014.01.012
50. Hachiya Y, Uruha A, Kasai-Yoshida E, Shimoda K, Satoh-Shirai I, Kumada S, et al. Rituximab ameliorates anti-N-methyl-D-aspartate receptor encephalitis by removal of short-lived plasmablasts. *J Neuroimmunol* (2013) 265(1-2):128–30. doi: 10.1016/j.jneuroim.2013.09.017
51. Henter ID, de Sousa RT, Zarate CA, Jr. Glutamatergic modulators in depression. *Harv Rev Psychiatry* (2018) 26(6):307–19. doi: 10.1097/HRP.0000000000000183
52. Posey DJ, Kem DL, Swiezy NB, Sweeten TL, Wiegand RE, McDougle CJ. A pilot study of D-cycloserine in subjects with autistic disorder. *Am J Psychiatry* (2004) 161(11):2115–7. doi: 10.1176/appi.ajp.161.11.2115
53. Urbano M, Okwara L, Manser P, Hartmann K, Herndon A, Deutsch SI. A trial of D-cycloserine to treat stereotypies in older adolescents and young adults with autism spectrum disorder. *Clin Neuropharmacol* (2014) 37(3):69–72. doi: 10.1097/WNF.0000000000000033
54. Urbano M, Okwara L, Manser P, Hartmann K, Deutsch SI. A trial of d-cycloserine to treat the social deficit in older adolescents and young adults with autism spectrum disorders. *J Neuropsychiatry Clin Neurosci* (2015) 27(2):133–8. doi: 10.1176/appi.neuropsych.13070155
55. Yang P, Lane H-Y, Hsu H-Y, Chang C-L. A pilot trial of sodium benzoate, a d-amino acid oxidase inhibitor, added on augmentative and alternative communication intervention for non-communicative children with autism spectrum disorders. *Transl Med (Sunnyvale)* (2017) 7:192. doi: 10.4172/2161-1025.1000192
56. Creten C, van der Zwaan S, Blankespoor RJ, Maatkamp A, Klinkenberg S, van Kranen-Mastenbroek VH, et al. Anti-NMDA-receptor encephalitis: a new axis-III disorder in the differential diagnosis of childhood disintegrative disorder, early onset schizophrenia and late onset autism. *Tijdschr Psychiatr* (2012) 54(5):475–9.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership