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The glutamate hypothesis of schizophrenia: evidence from human brain tissue studies

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Abstract

A number of studies have indicated that antagonists of the *N*-methyl-d-aspartate (NMDA) subtypes of glutamate receptors can cause schizophrenia-like symptoms in healthy individuals and exacerbate symptoms in individuals with schizophrenia. These findings have led to the glutamate hypothesis of schizophrenia. Here we review the evidence for this hypothesis in postmortem studies of brain tissue from individuals affected by schizophrenia, summarizing studies of glutamate neuron morphology, of expression of glutamate receptors and transporters, and of the synthesizing and metabolizing enzymes for glutamate and its co-agonists. We found consistent evidence of morphological alterations of dendrites of glutamatergic neurons in the cerebral cortex of subjects with schizophrenia and of reduced levels of the axon bouton marker synaptophysin. There were no consistent alterations of mRNA expression of glutamate receptors, although there has been limited study of the corresponding proteins. Studies of the glutamate metabolic pathway have been limited, although there is some evidence that excitatory amino acid transporter-2, glutamine synthetase, and glutaminase have altered expression in schizophrenia. Future studies would benefit from additional direct examination of glutamatergic proteins. Further advances, such as selective testing of synaptic microdomains, cortical layers, and neuronal subtypes, may also be required to elucidate the nature of glutamate signaling impairments in schizophrenia.

Introduction

For more than two decades phencyclidine (PCP), ketamine, and other antagonists of the *N*-methyl-d-aspartate (NMDA) subtypes of glutamate receptors have been known to induce schizophrenia-like, positive, negative, and cognitive symptoms.^{1–4} Similarly, NMDA receptor antagonist administration in schizophrenia subjects exacerbates these symptoms for

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Conflicts of interest

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prolonged periods.^{5,6} These observations have formed the bedrock of the *glutamate hypothesis* of schizophrenia, which, most broadly, posits that dysfunction of glutamatergic neurotransmission may be involved in the etiology of schizophrenia.⁷

This hypothesis has motivated a large number of animal model studies that have provided substantial support. For example, NMDA receptor antagonists have been found to induce schizophrenia-associated phenotypes in non-human primates and rodents, such as cognitive and sensorimotor gating impairments, hyperlocomotion, and social impairments.⁸ Animal model studies have also shown that the application of NMDA receptor autoantibodies, which in human patients induce a schizophrenia-like syndrome that is completely resolved following dialysis of the antibodies,⁹ cause NMDA receptor internalization, decrease NMDA receptor-mediated currents, and impair learning and memory.¹⁰ Supporting findings have also arisen from animal models that reduce levels of the NMDA receptor co-agonist d-serine, in which impaired long-term potentiation, reduced dendritic spine density, reduced hippocampal volume, and impaired memory performance have been observed.¹¹ Though not yet firmly established, clinical trials in subjects with schizophrenia suggest that enhancing NMDA receptor function via increasing availability of co-agonists has some efficacy.⁷

These findings, however, raise an important question. Is there direct evidence of alterations to glutamate signaling within individuals with schizophrenia? One approach to answering this question has been to measure glutamate indices using magnetic resonance spectroscopy in affected individuals. A recent review of many such studies concluded that elevated, not reduced, tissue levels of glutamate indices are present in medial prefrontal cortex in medication-naïve and medication-free patients.¹² Another widely used approach has been evaluation of genetic variation in components of glutamate signaling pathways. Although initial studies of positional and functional candidate genes suggested that many schizophrenia risk variants would be located in glutamate signaling genes,¹³ these initial findings were not clearly supported in large scale assessments of common variants.¹⁴ More recent studies that have examined common genetic variation have found genome-wide significant evidence of support for a number of glutamate signaling genes, including GRIA1, GRIN2A, GRM3 and SRR.¹⁸¹ In contrast, recent studies of rare and de novo mutations suggest that mutations in signaling molecules downstream of glutamate receptors, rather than in the receptors themselves, may contribute to schizophrenia risk.^{15,16}

In the current review, we examine the evidence for alterations in components of glutamate circuits and signaling pathways, as assessed in studies of postmortem brain tissue obtained from individuals diagnosed with schizophrenia and schizoaffective disorder during life. We review evidence for structural alterations in glutamatergic neurons. In addition, we review studies of mRNA and protein expression of molecules involved in glutamate signaling, specifically glutamate receptors, glutamate transporters, glutamate synthesizing enzymes, and glutamate receptor co-agonists.

Structural alterations in glutamate neurons (Table 1)

Somal volume

Somal volume has been evaluated in ten studies, including eight cortical areas. Because some studies tested multiple areas, and/or multiple cortical layers within a region, in all fourteen comparisons between schizophrenia and control subjects have been reported. Somal volume was decreased in SZ relative to control subjects in six comparisons, unchanged relative to control subjects in seven, and greater than control subjects in one. Somal size of pyramidal neurons decreased by 9.2–14.2% in deep layer III of DLPFC in SZ.^{17,18} Rajkowska *et al.*¹⁹ reported a 7.1% decrease in somal volume when both pyramidal neurons and interneurons are counted. Studies also found that pyramidal neurons staining positive for neurofilament 200 or nonphosphorylated epitopes of neurofilament have either larger or unchanged somal size,^{18,20} possibly indicating that other pyramidal neuronal subpopulations contribute to the lower average somal volume. However, a potential confound of these latter studies is that the immunostaining protocol used to label neurofilament epitopes in these studies over-estimates somal sizes, especially in SZ.²¹

In deep layer III of the primary and association auditory cortices, somal volume of pyramidal neurons were also decreased by 10.4% and 13.1%, respectively.^{22,23} However, such a decrease was not present in layer V,²³ consistent with layer specificity. One study, with a smaller sample size, did not find somal size differences, counting all neurons in the planum temporale, a structure containing the auditory association cortex.²⁴ An important consideration here is that when all neurons are counted, a small difference in pyramidal neurons, if present, would be masked by inclusion of numbers of interneurons. Decreased somal volume is also found in the insula,²⁵ but not in other brain areas such as fusiform cortex, primary visual cortex, and inferior parietal lobe.^{19,26,27} Whether chronic antipsychotic exposure affects somal volume has not been studied in animal models.

Dendrite arborization

Dendrite extent has been evaluated in five studies, including five cortical areas, in which dendritic arbors were found to be reduced in SZ relative to control subjects in four regions and unchanged relative to control subjects in one region. Several studies have found decreased basilar dendrite length by 14–29% or dendrite number in the prefrontal cortex, especially layer III.^{28–31} However, the decrease was not found in the primary visual cortex.³¹ One study found no difference in layer V or VI³² in the prefrontal cortex but two other studies found a decrease in either dendritic field size²⁸ or dendrite number²⁹ in layer V. Black *et al.*²⁸ reported a 40% decrease in basilar dendritic field size, a measure of both dendrite length and branching, in layer V pyramidal neurons. Whether chronic antipsychotic exposure affects dendrite arborization has not been studied in animal models.

Dendritic spine density

Dendritic spine density has been evaluated in five studies, including tissue from thirteen cortical areas in six brain regions (dorsolateral prefrontal cortex, orbitofrontal cortex, temporal association cortex, auditory cortex, hippocampal formation, visual cortex). Spine density was reduced in SZ relative to control subjects in five regions and unchanged relative

to control subjects in one. Decreases in spine density have been found in layer III of prefrontal, temporal, primary auditory, and auditory association cortices,^{31,33–35} but not in the primary visual cortex.³¹ The reported decreases ranged from 22–66% (median 27%) and included both estimates of decreased density of spines per dendritic length^{31,33,34} and decreased density of spines per μm^3 of gray matter.³⁵ In the one study evaluating deeper layers there was no decrease found in layers V or VI in prefrontal cortex.³² One study found a 35% decrease in spine density in the subiculum.³⁴ However, dendritic spine density was not altered either in animal models with antipsychotic exposure³⁵ or in human subjects with a non-schizophrenia psychiatric illness exposed to antipsychotics,³¹ suggesting the reduction in spine density observed in human SZ subjects was not related to antipsychotic use.

Axon boutons

Synaptophysin is a 38-kd synaptic vesicles protein present in virtually all presynaptic boutons.^{36–38} As a general marker of synapses, synaptophysin protein expression has been shown to reliably measure synaptic density.^{39–41} Synaptophysin protein expression has been evaluated in sixteen studies, including sixteen cortical areas. Because some studies tested multiple areas, in all twenty-seven comparisons between schizophrenia and control subjects have been reported. Synaptophysin protein expression was decreased in SZ relative to control subjects in twelve studies, unchanged relative to control subjects in fourteen studies, and greater than control subjects in one study. Decreases in synaptophysin protein expression have been found in frontal cortex in multiple studies, and in temporal and cingulate cortices in some studies.^{42–48} However, other studies do not find reductions in the same regions.^{49–54} Chronic antipsychotic exposure does not appear to affect synaptophysin expression in animal models.⁵⁵

The potential reduction in synaptophysin protein expression could reflect reduced synaptophysin protein per bouton, reduced density of axon boutons, or their combination. Interestingly, Sweet *et al.*⁵⁵ found that in deep layer III, but not layer I, of the primary auditory cortex the density of synaptophysin-immunoreactive puncta, i.e., presumptive axon boutons, was decreased by 14%. This reduction did not extend to the nearby auditory association cortex.⁵⁵ However, presumptive axon boutons labeled with antibodies directed against vesicular glutamate transporter 1 (VGluT1) and VGluT2, indicative of excitatory intracortical and thalamocortical axon boutons, respectively, were not altered in density or number in deep layer III of the primary auditory cortex in SZ.⁵⁶ This combination of findings—no change in VGluT-immunoreactive bouton density in the presence of reduced density of synaptophysin-immunoreactive bouton density—suggests one of two interpretations: either there are reductions in the density of non-glutamatergic boutons labeled by synaptophysin or synaptophysin protein levels within glutamate boutons is reduced (leading to reduced detectability of boutons when only labeled with synaptophysin). The latter alternative would have important implications in disease, as it would suggest that glutamatergic boutons are functionally impaired but structurally preserved, rendering them potential targets for treatments designed to enhance their function.

Molecular alterations in glutamate receptors

mRNA expression of glutamate receptors (Table 2)

Glutamate receptors are classified into two functional groups, *ionotropic* receptors, including NMDA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors, and *metabotropic* receptors.⁵⁷ NMDA receptors are tetramers, composed of 2 obligatory GRIN1 subunits and two regulatory GRIN2 or GRIN3 subunits.⁵⁸ AMPA receptors are tetramers, encoded by four genes (*GRIA1–4*). The receptors are assembled as dimers of homodimers of subunits.⁵⁸ Kainate receptors, encoded by five genes (*GRIK1–5*), are primarily located presynaptically and modulate synaptic activities.⁵⁹ Metabotropic glutamate receptors, which are coupled with G proteins, are encoded by eight genes.⁶⁰

Messenger RNA abundance has been studied by in situ hybridization, northern blotting, quantitative PCR, and other techniques, either individually or with microarray analysis that measures up to the entire transcriptome simultaneously. A number of microarray studies, which usually report genes with top changes, have been used to identify differentially expressed genes in SZ (reviewed in Sequeira *et al.*⁶¹). Genes involved in the glutamate neurotransmission pathway have been reported; however, glutamate receptor genes themselves generally do not stand out.⁶¹ It is to be remembered that microarray analysis is a screening test with false positives and false negatives and therefore results generally need to be verified with other methods. Individual glutamate receptor mRNA expression has been studied extensively in various brain areas. Changes in expression of glutamate receptors in SZ were previously reviewed by Rubio *et al.*⁶² and here we have also included new studies, as described below.

Several studies found decreases in mRNA expression of NMDA receptor subunits, such as GRIN1, GRIN2A, and GRIN2C, in the frontal cortex of schizophrenia subjects.^{63–65} However, this was not verified by other studies.^{66–69} One study reported increases in GRIN3A expression in schizophrenia,⁷⁰ while another study involving more subjects reported no increased expression.⁶⁵ Two studies found decreases in GRIA1, GRIA2, or GRIA4 mRNA expression in SZ,^{64,71} which were not found by two other studies.^{72,73} Decreases in expression of GRIK1,⁷⁴ GRIK3, GRIK4,⁶⁴ and GRIK5⁷⁵ have each been shown in only one of three studies above. One study reported increases in mGluR1 α mRNA expression in the PFC in SZ⁷⁶ and another study found increases in mGluR5 expression in BA11, but not in other prefrontal cortices such as BA9 and BA10.⁷⁷ One study found decreases in mGluR2 expression⁷⁸ in SZ, while another study showed no difference.⁷⁹

In the temporal cortex, decreases in mRNA expression of GRIN1,⁸⁰ GRIA2,⁸¹ or GRIK1⁸² have been reported in single studies, but not by others.^{66,82} In the occipital cortex, increases in mRNA expression of GRIN1 and GRIN2 have been reported,⁶⁷ which were not found by another study.⁶⁸ Increases in GRIA4 expression have been reported in one study,⁷² but not another.⁷³ In the hippocampus, decreases in GRIN1, GRIA1, and GRIA2 have been reported in two studies,^{81,83–86} while decreases in GRIK3 and GRIK5 were reported in a single study.⁸⁷ For GRIK1, one study reported a finding of decreased expression in the perirhinal cortex.⁸² In the thalamus, lower expression of NMDA, AMPA, or GRIK receptor subunits

has been reported in one or two studies,^{88–90} but not verified by others.^{91–93} Two studies found no change in MGLURs.^{90,93}

In contrast to structural studies indicating impairments of glutamatergic pyramidal neurons, the results above do not indicate any clear change in transcriptional control of glutamate receptor subunits in schizophrenia. For example, expression of the most studied mRNA, the GRIN1 subunit of the NMDA receptor, was reduced in four studies, unchanged in five studies, and increased in three of twelve studies. The lack of consistent findings could reflect a true negative. Other possibilities remain open, however. For example, studies of tissue homogenates, which include glutamate receptor RNA obtained from both pyramidal neurons and interneurons, might obscure findings limited to just one of those populations. Variable results could also reflect cohort-specific factors, including technical factors that affect RNA expression (see, for example, Sequeira *et al.*⁶¹ for a discussion of these issues). To the extent that functional changes in glutamate signaling ultimately require alterations in synaptic receptor protein levels, an alternative approach would be to assay receptor proteins directly.

Protein expression of glutamate receptors (Table 3)

There are fewer studies of glutamate receptors in SZ at the protein level than of mRNA, and these, generally speaking, are more recent than mRNA studies. Similar to the mRNA data, however, no clear trends have emerged. Kristiansen *et al.*⁹⁴ found no changes in expression of NMDA receptor subunits (GRIN1, GRIN2A–2D) in either the DLPFC or the ACC, except the C2' isoform of GRIN1 was higher in the ACC of SZ subjects. Rao *et al.*⁴⁸ found that GRIN1 and GRIN2B were unchanged in the DLPFC in SZ. In contrast to Kristiansen, Weickert *et al.*⁶⁵ showed that GRIN1 expression was lower in SZ in a larger study of an Australian sample. One study showed that GRIA1 and GRIA2 were lower in the DLPFC in SZ.⁹⁵ Using an antibody that identifies both mGluR2 and 3, Crook *et al.*⁹⁶ reported no differences in the DLPFC in SZ, but Gupta *et al.*⁹⁷ reported increases of both mGluR1α (consistent with an mRNA study⁷⁶) and mGluR2/3 in SZ. However, using subtype-specific antibodies, Ghose *et al.*⁹⁸ reported decreases in mGluR3 but no changes in mGluR2. Three studies reported no changes in mGluR5 expression.^{95,97,99} We found that in animal models chronic antipsychotic exposure did not appear to affect expression of some of the receptor subunits, such as GRIA3 and GRIA4.¹⁰⁰

Molecular alterations in glutamate transport, synthesis, and co-agonists (Table 4)

Vesicular glutamate transporter (VGluT) is involved in packaging of glutamate in synaptic vesicles.¹⁰¹ VGluT1 is preferentially expressed in neurons from the cortex and hippocampus, VGluT2 in neurons from subcortical structures.^{101–103} It has been reported that VGluT expression level correlates with synaptic glutamate release.¹⁰⁴ Eastwood and Harrison¹⁰⁵ found decreased VGluT1 mRNA expression in the prefrontal cortex and hippocampus in SZ; however, two other studies reported no such changes in the prefrontal cortex.^{106,107} In contrast, increased VGluT1 mRNA and protein was found in the ACC.¹⁰⁷ Oni-Orisan *et al.*¹⁰⁷ also found VGluT2 mRNA and protein levels unchanged in the ACC and PFC. Shan *et al.*¹⁰⁸ found no changes in either VGluT1 or VGluT2 protein expression in the superior temporal gyrus and hippocampus. Consistent with that report, Moyer *et al.*⁵⁶ found no differences in the number of VGluT1 and VGluT2 containing axon boutons or in

the mean within-bouton levels of VGluT1 and VGluT2 protein in the primary auditory cortex located in the superior temporal gyrus. Uezato *et al.*¹⁰⁹ also found no differences in expression of VGluT1 and VGluT2 mRNA in the medial temporal lobe, although they reported that VGluT2 mRNA expression was lower in the inferior temporal gyrus in SZ. However, in the inner molecular layer of the dentate gyrus, Talbot *et al.*¹¹⁰ found that VGluT1 protein expression was increased.

Glutamate is eliminated from the synaptic cleft by excitatory amino acid transporters (EAATs).¹¹¹ EAAT1 and 2 are expressed primarily in glial cells,^{112,113} while EAAT3 and 4 are primarily expressed by neurons.^{114–116} Around 90% of glutamate at the synaptic cleft is cleared by EAAT2.^{117,118} Thus, changes in EAAT expression can potentially alter synaptic glutamate levels. Bauer *et al.*¹¹⁹ found that in SZ EAAT1 protein expression was lower in the DLPFC, EAAT2 was unchanged, and EAAT3 was higher in the ACC but not in the DLPFC. Bauer *et al.*¹²⁰ later found less glycosylation of EAAT1 and 2 in SZ, a process important for plasma membrane localization. The same group also found lower protein expression of EAAT1 and 2 in the superior temporal gyrus, lower EAAT2 in the hippocampus, with EAAT3 level unchanged.¹⁰⁸ Rao⁶⁹ reported that EAAT1, EAAT3, and EAAT4 protein expression were higher, while EAAT2 was unchanged in the DLPFC in SZ. Therefore, conflicting results have been reported from studies by the two groups, although both found no changes of EAAT2 in the DLPFC.

After synaptic uptake into astrocytes by EAATs, glutamate is turned into glutamine by glutamine synthetase (GS).^{121,122} Glutamine is subsequently delivered to neurons where it is turned into glutamate by phosphate-activated glutaminase (PAG). Therefore, GS and PAG expression levels can potentially affect glutamate cycling and availability to neurons. Katsel *et al.*¹²³ found that GS mRNA expression was lower in the deep, but not superficial, layers of the ACC, indicating layer specificity. Steffek *et al.*¹²⁴ found that GS protein expression was lower in the superior temporal gyrus (STG) and ACC, but not in the DLPFC, primary visual cortex, or hippocampus of subjects with SZ. However, two other studies showed decreased GS protein level in the frontal cortex in SZ,^{125,126} while a third study was negative.¹²⁷ One study found that GS protein was higher in the ACC of female SZ patients only, not in males.¹²⁸ In addition, Gluck¹²⁹ found that GS enzymatic activity was unchanged in the DLPFC in SZ. Only a few postmortem studies investigated PAG in SZ. Bruneau *et al.*¹³⁰ found that transcripts of both PAG and glutamine synthetase were increased in the thalamus of SZ subjects. Katsel *et al.*¹²³ found PAG transcript was unchanged in the ACC of SZ subjects. One study found that PAG activity was 4-fold greater in the DLPFC of subjects with SZ.¹²⁹

D-Serine, an NMDA receptor co-agonist,¹³¹ is synthesized by serine racemase from l-serine,^{132,133} and it can be degraded by d-amino acid oxidase (DAAO).^{134,135} Labrie *et al.*¹³⁶ reviewed d-serine in schizophrenia, and found some evidence of decreased levels of d-serine or ratio of d-serine to total serine in serum or cerebrospinal fluid. Studies have shown decreased,¹³⁷ unchanged,¹³⁸ and increased levels of serine racemase^{139,140} in SZ. DAAO expression was higher in the hindbrain than forebrain.^{141,142} Increased DAAO expression and activity have been reported in the cerebellum in SZ.^{138,140} However, most studies in the cortex were negative^{137,138,140} although one study did show increased DAAO activity in the

parietal cortex in SZ.¹⁴³ Kynurenic acid (KYNA), a metabolite of kynurenine arising in the tryptophan degradation pathway, is an antagonist at the glycine site of NMDA receptors.¹⁴⁴ KYNA levels were reported higher in the prefrontal cortex¹⁴⁵ and cerebrospinal fluid¹⁴⁶ of SZ patients. Kynurenine 3-monooxygenase (KMO) decreases KYNA synthesis by metabolizing kynurenine into products other than KYNA.¹⁴⁷ In one study, Wonodi *et al.*¹⁴⁷ found that KMO expression and enzymatic activity were lower in the frontal eye field of the cortex, potentially contributing to a higher level of KYNA. Kynurenine aminotransferase-1, involved in KYNA synthesis, was found to have higher enzyme activity in the cerebellum without change in mRNA expression.¹³⁸

Conclusions and recommendations for future progress

The glutamate hypothesis of schizophrenia has been elaborated largely on the basis of strong support from pharmacologic challenge studies in humans and in animal models, although other evidence, such as the ability of anti-NMDA receptor autoantibodies to induce psychotic symptoms, is also supportive. In the current review we evaluated whether there is direct evidence of glutamatergic impairments in brain tissue obtained from individuals who experienced schizophrenia during life. These studies reveal several clear conclusions. First, there is consistent evidence for morphological alterations of dendrites of glutamatergic neurons in the cerebral cortex of subjects with schizophrenia, and of reduced levels of the axon bouton marker, synaptophysin. Second, despite their expression in these affected structures, there is no clear evidence for reduction of mRNA expression of glutamate receptors and vesicular transporters, although there has been limited study of the corresponding proteins. Third, while the number of studies of molecules regulating the metabolism of glutamate and its co-agonists has been limited, there is some evidence that the key components of the glutamate metabolic cycle, EAAT2, glutamine synthetase, and glutaminase, have altered expression in schizophrenia.

The study of glutamatergic, or other alterations, in postmortem brain tissue from affected individuals has several distinctive advantages and limitations that need to be considered in order to evaluate the findings above. Although *in vivo* imaging methods are expanding the number of molecules that can be investigated, at present brain tissue studies are the only approach by which alterations in large numbers of molecules within specific cerebral cortex layers, cells, and cellular compartments can be detected. With regard to brain tissue studies in affected individuals, to the extent that schizophrenia is an illness of the brain, the relevant pathologies leading to schizophrenia must be manifest, and therefore detectable, in the circuits, cells, cellular compartments, and molecules within the brains of affected individuals. Importantly, for a number of reasons it cannot be assumed that the same pathologies can be more readily created and evaluated in animal models. Although there is substantial conservation of neuronal genes, proteins and cell types from mice to men, the differences in molecules, architecture, and connectivity can have substantial impact. This has been very evident in the study of Alzheimer's disease, where small amino acid sequence differences in mouse amyloid- β protein prevent it from aggregating into the toxic assemblies present in human.¹⁴⁸ Even when transgenic human amyloid- β sequences containing mutations that cause Alzheimer's disease in humans are introduced into mice, many key aspects of the neurodegenerative pathology are not reproduced.¹⁴⁸ It is not yet clear if the

advent of transgenic approaches in other mammals will solve these problems for some brain disorders.¹⁴⁹ More recently, an intriguing observation indicated that there is a high rate of somatic mosaicism (i.e., the presence of genetic variation not transmitted through the germ line) among human neurons.¹⁵⁰ If confirmed, one implication would be that it may be only possible to study the molecular manifestations of human brain disease in human brain tissue itself.

There are also disadvantages to the use of human brain tissue to study a chronic disease with onset early in life, such as schizophrenia. Many of these factors are technical in nature, and can be mitigated with rigorous attention to the methodology for tissue harvesting, preservation, characterization, and diagnostic evaluation.¹⁵¹ However, a fundamental limitation arises from the fact that most individuals who die with disease do so years, and more commonly decades, after onset. Thus, aging itself, which can affect neuronal morphology and gene expression, might impact the differences between SZ and normal subjects (i.e., an age by disease interaction). The mean ages of subjects included in the studies summarized above vary broadly from mid-life to late-life. However, the many additional cohort and technical factors that differ between reports are confounded by differences in subject ages, precluding any conclusion that differences between studies can be reconciled as due to an interaction of aging with the disease process.

In addition to possible effects of aging, the long illness durations of most subjects prior to death make it difficult to disentangle those alterations that may have contributed to the cause and persistence of symptoms of schizophrenia from those that may be either compensatory or the result of chronic disease, of co-morbid conditions such as nicotine and other substance use, or of long term medication treatment. It is possible in some cases to evaluate the impact of substances and medications via construction of appropriate contrasts among human subjects in post-mortem studies. However, post-mortem human studies are not that useful for assessing long term effects of antipsychotics because the vast majority of subjects have received such treatment for a long period of time. The best alternative approach to evaluate this effect in tissue studies is to use animal models chronically treated with antipsychotics, as described above, e.g., regarding the effect of antipsychotic exposure on dendritic spine density and axon boutons). Nevertheless, the problem of cause versus compensation often remains problematic. This concern can be seen in the findings regarding components of the glutamate cycle, in which postmortem tissue studies suggest reductions in EAAT2 and glutamine synthetase, and increased PAG. These findings might predict indices of glutamate would be altered *in vivo* in chronic subjects with schizophrenia. In contrast, *in vivo* studies suggest overall that tissue levels of glutamate indices are unchanged in chronic, treated schizophrenia, but instead are elevated at first episode of illness.¹² This raises the possibility that the postmortem findings reflect a compensation in the face of an earlier, sustained glutamate elevation or even a medication effect, although animal model studies largely do not indicate an effect of long term antipsychotic treatment.^{108,123,124,152} However, it should be noted that the relative contribution of neuronal somata, neuronal presynaptic boutons, astrocytes, and extracellular glutamate to the tissue levels of glutamate indices detected by *in vivo* MRS is not known.

One approach to overcome most of the above concerns would be to develop a sufficiently large collection of samples from individuals who died very early in disease- a necessary but challenging enterprise. Alternatively, an approach is to relate identified alterations in postmortem brain tissue to potentially causal genetic variations (ideally mutations of moderate to high penetrance). For example, we recently reported reduced protein levels of ATP1A3 in auditory cortex of subjects with schizophrenia.¹⁰⁰ Rare mutations in *ATP1A3* have been identified as contributing to a polygenic burden for schizophrenia risk,¹⁶ and mutations in *ATP1A3* that reduce protein expression are also strongly linked to rapid-onset dystonia-parkinsonism, a movement disorder in which individuals with *ATP1A3* mutations also display significant impairments in memory, attention, and executive function, and in which 19% develop a psychotic syndrome characterized by auditory hallucinations prior to or at onset of the motor symptoms.¹⁵³ Thus, the genetic findings strongly support the interpretation that the altered ATP1A3 expression in postmortem brain could contribute to onset of symptoms (rather than result from chronic illness), although confirmatory studies in model systems are also needed.

Another potential technical issue affecting postmortem studies that attempt to identify the molecular correlates of the structural alterations in glutamate neurons in schizophrenia brain tissue has been the preponderance of studies measuring mRNA expression. As summarized in Table 2, investigation of mRNA transcript levels of the glutamate receptors found in specific brain structures has not provided clear consensus on the extent of change (if any) or direction of change between disease and control tissues. The lack of correspondence between the structural and molecular studies could be one of spatial resolution. One advantage histological studies have over many mRNA studies is the ability of the former to resolve the physical unit being quantified, e.g, dendritic spines. The ability to differentiate between cortical layers, neuronal subtypes, and synaptic compartments has been essential to the successful detection of alterations in neuronal morphology. Thus, differences measured in mRNAs between schizophrenia and control tissues localized to a specific layer, neuronal type, or synaptic compartment could be lost in the noise of a homogenized tissue sample. The use of in situ hybridization and, more recently, the introduction of laser-capture and linear amplification strategies have mitigated this issue somewhat, allowing for quantification of mRNAs in discrete cortical layers and specific neuronal populations.^{90,154,155}

Some of the variability in the molecular findings might also reflect the more general problem that mRNA levels explain only a minority of the variance in protein level for many proteins.¹⁵⁶ Unfortunately, to date only limited analysis of glutamate receptor protein levels in schizophrenia has been reported, the number of evaluations too few to draw clear conclusions (Table 3). Moreover, the needed additional investigation of protein levels would benefit from an approach that advances traditional strategies (such as immunoblotting) with the ability to provide multiplex quantification so as to more comprehensively survey the glutamatergic protein network within individuals, to generate results with high precision and accuracy, and to have the necessary sensitivity for measurement of synaptic microdomains, cortical layers, and neuronal subtypes.

Mass spectrometry (MS) proteomic approaches have been used extensively to catalog protein expression in mammalian neuronal systems, and are beginning to see application in the investigation of human brain disease. MS approaches to protein analysis vary, but most workflows fall into one of three types: multi-dimensional protein identification technology (MudPIT), differential MS, and selected reaction monitoring. In MudPIT, proteins are cleaved in to predictable peptide components which are then separated into different fractions, to overcome the dynamic range of protein expression, often by reverse phase chromatography or SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). These fractions are then analyzed one at a time by high mass accuracy ion trap or time-of-flight instrumentation, to identify and quantify the tryptic peptides. In differential MS, the peptide fragments are instead quantified by ion trap or time-of-flight instrumentation without first being sequenced for identification. Peptides found to be differentially expressed are then sequenced for identification in subsequent analyses. In selected reaction monitoring, preselected peptides are targeted for quantification. The first two methods are capable of quantifying 1000s of proteins in complex mixtures but often have high tissue and instrument time requirements and are well suited for describing complex proteomes and hypothesis generation. In contrast to these approaches, selected reaction monitoring is capable of quantifying fewer proteins, 100's, but does have several notable advantages. First, it is not confounded by dynamic range of protein expression, thus sample enrichment/fractionation is often unnecessary and far less instrument time is required allowing for higher throughput. Second, it is extremely sensitive, and thus protein requirements can be as low as 10–100 nanograms. This allows for the analysis of biochemical fractions such as synaptic microdomains,¹⁵⁷ and should similarly facilitate approaches such as laser capture microdissection of cortical layers and potentially cell types. As new instrumentation is introduced the breadth, throughput, and sensitivity of these approaches will continue to advance allowing for more accurate and comprehensive investigations of glutamate signaling protein abnormalities in schizophrenia.

Despite these potential interpretive and technical issues, some conclusions about the postmortem findings regarding glutamatergic measures in schizophrenia are possible. As a whole, these studies reveal strong evidence for structural alterations of dendrites of glutamatergic neurons in schizophrenia, with reduced dendrite length and complexity, and reduced dendritic spine density each reported in multiple regions, from multiple cohorts, by multiple labs (Table 1). Although some reports found these parameters unchanged, opposing findings (i.e., increased dendritic measures) have not been reported. While not as consistent, reductions in pyramidal neuron somal volume have also been reported in many studies. When assessments of pyramidal neuron structure have been made across multiple cortical layers, alterations affecting layer III pyramidal neurons have been most commonly, but not exclusively, reported. Finally, both levels of the axon bouton marker synaptophysin (a protein that is predominantly, but not specifically, expressed within glutamatergic boutons in cerebral cortex) and synaptophysin immunoreactive puncta density are also widely reported to be decreased in schizophrenia (Table 1). While these structural alterations could represent consequences of long term illness, there is now a substantial body of longitudinal *in vivo* imaging evidence indicating that progressive reductions in cortical gray matter volume occur in subjects with schizophrenia around the onset of overt psychosis, suggesting these

structural alterations are an early disease-related alteration.¹⁵⁸ Similarly, animal model studies do not indicate that either dendritic spine or bouton loss is caused by long term antipsychotic exposure.^{35,55}

The findings of structural alterations in at least some cortical pyramidal neurons provides compelling support for the presence of glutamatergic alterations in schizophrenia, and provides some links to other elements of the glutamate hypothesis of schizophrenia. For example, it is well established that activity-dependent glutamate signaling has been shown to modify spine and dendrite structure.^{159–161} While it cannot be concluded that the observed alterations in pyramidal neuron structure in subjects with SZ arise as a result of impaired glutamate signaling, it is at least possible that they do, as there is evidence that disruptions at various points within the glutamate signaling pathway may cause alterations in pyramidal cell morphology, mimicking those found in SZ. For example, in mature neural systems, either pharmacological blockade of GRIAs¹⁶² or deafferentation of glutamatergic projections^{162–164} decreases density of spines. Reduction in NMDA receptor activity by knockout of serine racemase results in reduced dendritic length, branching, and spines.^{165,166}

Similarly, we have demonstrated reduced dendritic spine density and reduced pyramidal cell somal volume in layer III of the primary auditory cortex (AI). It is largely the reciprocal connections of these same layer III pyramidal cells in AI that sharpen frequency tuning to selectively enhance the preferred frequency during auditory perception,^{167,168} a necessary prerequisite for tone discrimination. Individuals with SZ demonstrate impairments of tone discrimination^{169,170} and of the generation of auditory event-related potentials that localize to AI, such as mismatch negativity (MMN).^{171,172} MMN and tone discrimination likely tap the same underlying intracortical mechanisms,¹⁷³ and the degree of impairment in MMN and tone discrimination are correlated.¹⁶⁹ Reductions of MMN similar to those in subjects with SZ can be modeled by infusing NMDA antagonists into the auditory cortex,¹⁷³ an observation paralleled by systemic administration of NMDA antagonists in mice¹⁷⁴ and in normal humans,¹⁷⁵ thus linking the observation of altered cortical pyramidal neuron structure to one of the key historical observations underlying the glutamate hypothesis of schizophrenia. Whether dendritic spine loss in itself functionally mimics the effects of NMDA receptor antagonism in intact tissue will require investigation in disease-relevant models of dendritic spine loss. Alternatively, whether the remaining dendritic spines in SZ subjects are additionally affected by deficits in glutamate receptors or signaling molecules that could be targeted therapeutically will benefit from additional studies of postmortem tissue that address some of the technical limitations reviewed above.

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Table 1

Summary of studies of structural alterations in glutamate (pyramidal) neurons

Reference	Brain region	SZ/Cntl	Methods	Difference in SZ
Somal volume				
19	BA9	9/10	Nissl	7% decrease in layer III
17	BA9	28/28	Nissl	9.2% decrease in deep layer III
18	BA9	13/13	Nissl	14.2% decrease in deep layer III
24	BA9	11/10	Nissl	No differences in layer IIIb-c
18	BA9	9/9	IHC	No difference in NNFP-IR neurons in deep layer III
20	BA9	11/13	IHC	59% increase in layer III NF200-IR pyramidal neuron volume
23	BA41	16/16	Nissl	10.4% decrease in deep layer III
22	BA42	18/18	Nissl	13.1% decrease in deep layer III
23	BA42	18/18	Nissl	No difference in layer V
24	PT (~BA41, BA42, BA22)	11/10	Nissl	No difference in layers II, IIIc, or VI
25	Insula (~BA13)	15/15	Nissl	16.2% decrease in layer II but not in layer III
26	Fusiform cortex (~BA37)	11/13	Nissl	No difference in pyramidal cells of layers III or V
27	~BA40	24/24	Nissl	No difference in upper or lower cortical layers or lower layer III
19	BA17	7/7	Nissl	No difference
Dendritic length and number				
31	BA46	15/15	Golgi	14% decrease in deep, but not superficial, layer III
32	BA 46	14/15	Golgi	No difference in layers V or VI
30	BA11	5/5	Golgi	29% decrease in layer III
28	BA10	15/18	Golgi	40% decrease in layer V
29	BA32	11/11	Golgi	29% and 46% decrease in primary and secondary dendrite numbers respectively in layer V; 17% and 15% decrease in layer III
31	BA17	13/15	Golgi	No difference in layer III
Dendritic spine density				
31	BA46	15/15	Golgi	23% decrease in deep, but not superficial, layer III
32	BA 46	14/15	Golgi	No difference in layers V and VI
33	BA10, BA11, BA45	13/11	Golgi	66% decrease in layer III
33	BA38, BA20, BA21, BA22	13/11	Golgi	59% decrease in layer III
35	BA41	15/15	IHC	27% decrease in spinophilin-IR puncta in deep layer III
35	BA42	15/15	IHC	22% decrease in spinophilin-IR puncta in deep layer III
34	Subiculum	13/8	Golgi	35% decrease
31	BA17	13/15	Golgi	No difference in layer III
Synaptophysin protein expression				
52	BA 8	10/13	IB	No difference
47	BA9	5/4	IB	Decrease
43	BA9	10/10	IHC	Decrease
50	BA9	19/19	IB, IAR	No difference

Reference	Brain region	SZ/Cntl	Methods	Difference in SZ
45	~BA10	13/10	ELISA	No difference
47	BA10	6/6	IB	Decrease
44	BA10	14/12	IB	Decreases
48	BA10	10/10	IB	Decrease
46	BA45, BA46	5/6	IB	Trend of decrease
53	PFC	18/23	IB	No difference
43	BA46	10/10	IHC	Decrease
50	BA46	19/19	IB, IAR	No difference
46	BA32, BA33	18/12	IB	Decrease
176	~BA32, BA33	11/13	IB	Decrease
54	~BA24	18/24	ELISA	No difference
51	BA24	15/14	IB	No difference
52	BA24	17/15	IB	Increase
42	Medial temporal lobe	11/14	IAR	Decrease
49	Medial temporal lobe	7/13	IR	No difference
46	Hippocampus	13/10	IB	Decrease
52	BA20	19/12	IB	No difference
47	BA20	6/4	IB	Decrease
46	BA21, BA22	13/9	IB	No difference
46	BA39, BA40	15/10	IB	Trend of decrease
52	BA7	8/12	IB	No difference
43	BA17	10/10	IHC	No difference
47	BA17	10/6	IB	Decrease
Axon boutons				
55	BA41	15/15	IHC	14% decreases of synaptophysin-IR puncta density in deep layer III
56	BA41	27/27	IHC	No differences in VGluT1-, VGluT2-IR bouton density or number in deep layer III
55	BA42	15/15	IHC	No differences in synaptophysin-IR puncta density

Abbreviations: SZ, schizophrenia; Cntl, control; BA, Brodmann area; PT, planum temporale; IHC, immunohistochemistry; IB, immunoblotting; ELISA, enzyme-linked immunosorbent assay; IR, immunoreactivity; IAR, immunoautoradiography; NNFP, nonphosphorylated neurofilament protein; NF200, neurofilament 200. ~indicates approximate region based on description of location in published report. Red, yellow, and green colors represent decreases, no changes, and increases respectively in structural measures in schizophrenia.

Table 2

Summary of studies of mRNA expression of glutamate receptors in brain regions in schizophrenia

Region (Reference)	n (SZ/Cnt)	NMDA						AMPA				Kainate					Metabotropic				
Frontal cortex		GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIN3A	GRIA1	GRIA2	GRIA3	GRIA4	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	MGLUR1	MGLUR2	MGLUR3	MGLUR4	MGLUR5
66	15/15																				
64	21/9	&												&	&						
73	16/9																				
77	6/10																				
68	6/6																				
67	10/10																				
75	15/9																				
70	15/15																				
72	36/26																				
74	20/20																				
71	15/15																				
63	15/15																				
155	15/15																				
79	14/23																				
78	7/7																				
76	42/42																				
48	10/10																				
65	37/37																				
Temporal cortex		GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIN3A	GRIA1	GRIA2	GRIA3	GRIA4	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	MGLUR1	MGLUR2	MGLUR3	MGLUR4	MGLUR5
81	9/14																				
80	8/7	#																			
66	15/15																				
68	6/6	##																			
70	15/15																				
82	15/15																				

Region (Reference)		NMDA						AMPA				Kainate					Metabotropic				
	n (SZ/CntI)	GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIN3A	GRIA1	GRIA2	GRIA3	GRIA4	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	MGLUR1	MGLUR2	MGLUR3	MGLUR4	MGLUR5
Frontal cortex		GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIN3A	GRIA1	GRIA2	GRIA3	GRIA4	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	MGLUR1	MGLUR2	MGLUR3	MGLUR4	MGLUR5
Occipital cortex		GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIN3A	GRIA1	GRIA2	GRIA3	GRIA4	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	MGLUR1	MGLUR2	MGLUR3	MGLUR4	MGLUR5
73	16/9																				
68	6/6																				
75	15/9																				
67	10/10																				
72	36/26																				
Hippocampus		GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIN3A	GRIA1	GRIA2	GRIA3	GRIA4	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	MGLUR1	MGLUR2	MGLUR3	MGLUR4	MGLUR5
85	6/8																				
81	9/14																				
87	11/13																				
83	11/11																				
84	24/21 for NR1, 8/4 for NR2A, 2B																				
82	15/15																				
86	30/31																				
Thalamus		GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIN3A	GRIA1	GRIA2	GRIA3	GRIA4	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	MGLUR1	MGLUR2	MGLUR3	MGLUR4	MGLUR5
93	12/8																				
89	12/8																				
88	13/8																				
91	15/15																				
92	14/16																				
90	14/20																				

& significant difference was found only in SZ subjects who were neuroleptic free >6 months, not in those receiving neuroleptic until death;

& white matter only;

& exon 22 containing NR1 splice isoform;

* pyramidal cell layers of BA11 only;

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MGLUR1alpha only;

there was no change in total NR1 level, while the NR1-2b splice form was decreased in the right hippocampus and the NR1-4b splice form was decreased in the left hippocampus;
GRIN1 was decreased only in cognitively impaired SZ subjects.
the NR1 splice isoform containing neither C-terminal C1 nor C2 cassette only;
relay neurons of medial dorsal thalamus only.
Red, yellow and green colors represent decreases, no changes, and increases respectively in gene expression in schizophrenia.

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Table 3

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Summary of studies of protein expression of glutamate receptors in brain regions in schizophrenia

Region (Reference)	n (SZ/Cntl)	NMDA					AMPA				Kainate					Metabotropic				
		GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIA1	GRIA2	GRIA3	GRIA4	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	MGLUR1	MGLUR2	MGLUR3	MGLUR4	MGLUR5
Hippocampus ¹⁷⁷	13/8																			
Hippocampus ¹⁷⁸	11/10																			
Hippocampus ¹⁷⁹	15/15																			
DLPFC ⁹⁶	20/20																			
PFC ⁹⁷	16/9																			
DLPFC ⁹⁴	13/7																			
ACC ⁹⁴	13/7																			
Thalamus ¹⁸⁰	15/8																			
DLPFC ⁹⁸	15/15																			
TC ⁹⁸	15/15																			
MC ⁹⁸	15/15																			
Hippocampus ⁸⁶	8/7																			
BA10 ⁹⁵	21/35																			
DLPFC ⁴⁸	10/10																			
DLPFC ⁹⁹	37/37																			
DLPFC ⁶⁵	37/37																			
BA41 ¹⁰⁰	22/23																			

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~ single antibody raised against common epitope of GRIA2 and GRIA3;
* MGLUR1 alpha only;
** C2' isoform only;
*** expression was lower in the left hippocampus of male subjects only.
single antibody against GRIK1/2/3;
single antibody against both MGLUR2 and MGLUR3;
dorsomedial thalamus only;

Abbreviations: DLPFC, dorsolateral prefrontal cortex; PFC, prefrontal cortex; ACC, anterior cingulate cortex; TC, temporal cortex, MC, motor cortex. Red, yellow and green colors represent decreases, no changes, and increases respectively in gene expression in schizophrenia, 4a, MGLUR4 subtype alpha.

Table 4

Summary of alterations in glutamate transport, synthesis, and co-agonist in brain regions in schizophrenia

Reference	Brain region	n (SZ/Cnt)	Methods	Difference in SZ			
				EAAT1	EAAT2	EAAT3	EAAT4
EAATs							
77	BA9, BA10	6/10	mRNA				
119	DLPFC	20/11	mRNA				
119	DLPFC	13/8	protein	monomer			
119	ACC	20/11	mRNA				
119	ACC	13/8	protein			multimer	
69	BA10	10/10	mRNA				
69	BA10	10/10	protein				
108	STG	23/27	protein				
108	HC	23/27	protein				
120	DLPFC	33/32	glycosylation		multimer		
120	ACC	34/34	glycosylation	monomer			
VGluT				VGluT1		VGluT2	
110	DGiml	17/17	protein				
105	HC	13/12	mRNA				
105	DLPFC	10/10	mRNA				
105	STG	11/12	mRNA				
107	DLPFC	18/11	mRNA				
107	ACC	18/11	mRNA				
107	DLPFC	23/27	protein				
107	ACC	23/27	protein				
109	HC, EC, MTG	13/13	mRNA				
109	ITG	13/13	mRNA				
106	DLPFC	37/37	mRNA				
108	HC	23/27	protein				
108	STG	23/27	protein				

Reference	Brain region	n (SZ/Cntl)	Methods	Difference in SZ			
				EAAT1	EAAT2	EAAT3	EAAT4
EAAT's							
56	Deep layer 3 of primary auditory cortex	27/27	protein (IHC)				
Glutamine synthetase							
129	DLPFC	27/13	enzymatic activity				
125	Prefrontal cortex	8/9	protein				
126	Prefrontal cortex	10/10	protein				
130	Thalamus	13/8	mRNA				
127	PFC	15/15	protein				
124	ACC, STG	23/27	protein				
124	DLPFC, PVC, HC	23/27	protein				
128	ACC	11/8	protein				
123	Deep layers of ACC	18/21	mRNA				
Phosphate-activated glutaminase							
129	DLPFC	27/13	enzyme activity				
130	Thalamus	13/8	mRNA				
123	ACC	18/21	mRNA				
Serine racemase							
138	Cerebellum, parietal cortex	4/5	mRNA				
139	HC	23/27	protein				
139	DLPFC, ACC, STG PVC	23/27	protein				
140	Cerebellum	15/15	mRNA and protein				
140	DPFC	15/15	mRNA				
140	DPFC	15/15	protein				
137	BA9	13/14	protein				
137	HC	15/14	protein				
DAO							
138	cerebellum	4/5	mRNA and enzyme activity				
140	cerebellum	14/14	mRNA				

Reference	Brain region	n (SZ/Cntd)	Methods	Difference in SZ			
				EAAT1	EAAT2	EAAT3	EAAT4
EAAT's							
140	cerebellum	14/14	protein		Trend of increase		
138	Parietal cortex	4/5	mRNA				
140	DPFC	14/14	mRNA				
137	BA9 and HC	15/14-15	protein				
143	Parietal cortex	15/15	enzyme activity				
KMO							
147	BA6	32/32	mRNA and enzyme activity				
KAT-1							
138	cerebellum	4/5	Enzyme activity				
138	cerebellum	4/5	mRNA				

* only hDAAOI isoform mRNA was increased in SZ without a change in total DAAO mRNA.

Abbreviations: DPFC, dorsal prefrontal cortex; DLPFC, dorsolateral prefrontal cortex; ACC, anterior cingulate cortex; HC, hippocampus; EC, entorhinal cortex; DGImI, the inner molecular layer of the dentate gyrus; PVC, primary visual cortex; STG, superior temporal gyrus; ITG, inferior temporal gyrus; MTG, middle temporal gyrus; DAAO, D-amino acid oxidase; KMO, kynurenine 3-monooxygenase; KAT-1, kynurenine aminotransferase-1. Red, yellow and green colors represent decreases, no changes, and increases respectively in schizophrenia.