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Altered Gradients of Glutamate and Gamma-Aminobutyric Acid Transcripts in the Cortical Visuospatial Working Memory Network in Schizophrenia

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ABSTRACT

BACKGROUND: Visuospatial working memory (vsWM), which is impaired in schizophrenia, requires information transfer across multiple nodes in the cerebral cortex, including visual, posterior parietal, and dorsolateral prefrontal regions. Information is conveyed across these regions via the excitatory projections of glutamatergic pyramidal neurons located in layer 3, whose activity is modulated by local inhibitory gamma-aminobutyric acidergic (GABAergic) neurons. Key properties of these neurons differ across these cortical regions. Consequently, in schizophrenia, alterations in the expression of gene products regulating these properties could disrupt vsWM function in different ways, depending on the region(s) affected.

METHODS: Here, we quantified the expression of markers of glutamate and GABA neurotransmission selectively in layer 3 of four cortical regions in the vsWM network from 20 matched pairs of schizophrenia and unaffected comparison subjects.

RESULTS: In comparison subjects, levels of glutamate transcripts tended to increase, whereas GABA transcript levels tended to decrease, from caudal to rostral, across cortical regions of the vsWM network. Composite measures across all transcripts revealed a significant effect of region, with the glutamate measure lowest in the primary visual cortex and highest in the dorsolateral prefrontal cortex, whereas the GABA measure showed the opposite pattern. In schizophrenia subjects, the expression levels of many of these transcripts were altered. However, this disease effect differed across regions, such that the caudal-to-rostral increase in the glutamate measure was blunted and the caudal-to-rostral decline in the GABA measure was enhanced in the illness.

CONCLUSIONS: Differential alterations in layer 3 glutamate and GABA neurotransmission across cortical regions may contribute to vsWM deficits in schizophrenia.

Keywords: GABA, Glutamate, Prefrontal cortex, Schizophrenia, Visual cortex, Working memory

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Long-term functional outcomes in schizophrenia are largely determined by the severity of cognitive impairments (1,2). These impairments include disturbances in visuospatial working memory (vsWM) (3), the ability to transiently maintain and manipulate visuospatial information to guide thought and behavior (4). Deficits in vsWM not only characterize individuals with schizophrenia but also may predict transition to psychosis in prodromal individuals (5).

In primates, vsWM is mediated by a distributed cortical network that includes nodes in the primary visual cortex (V1) and association visual cortex (V2) of the occipital lobe, which convey visual information to nodes in the posterior parietal cortex (PPC) and dorsolateral prefrontal cortex (DLPFC) (6–8). This feedforward information is principally carried by excitatory projections from glutamatergic layer 3 pyramidal neurons in each region (9). Within each region, the activity of layer 3 pyramidal neurons during vsWM tasks is shaped by local inhibitory, gamma-aminobutyric acid (GABA) neurons (10,11).

Key elements regulating glutamatergic and GABAergic neurotransmission differ across regions in the vsWM network. For example, patterns of gene expression in cortical gray matter exhibit a caudal-to-rostral gradient, with the V1 and DLPFC at opposite ends of this gradient (12–15). These patterns include lower levels of the glutamate ionotropic receptor alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid type subunit 2 (GRIA2) in the V1 relative to the DLPFC (16), and higher levels of the $GABA_A$ receptor $\alpha 1$ subunit in the V1 than in the DLPFC (17). Similarly, the properties of layer 3 pyramidal and GABA neurons also exhibit regional differences. For example, layer 3 pyramidal neurons in the V1 have lower dendritic spine densities, have smaller soma sizes, and are more excitable compared with those in the DLPFC (18,19). Together, these findings suggest that markers of glutamate

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and GABA neurotransmission in layer 3 are likely to differ across the cortical regions that mediate vsWM function.

Thus, in schizophrenia, alterations in these markers could disrupt vsWM function in different ways, depending on the regions affected. Markers of glutamate neurotransmission have been examined in schizophrenia, but findings are inconsistent across studies and cortical regions (20). In contrast, consistent disease-related alterations in markers of cortical GABA neurotransmission have been reported (17,21,22), although most studies focused on the DLPFC (23). The few studies that examined GABA markers in multiple brain regions within the same subjects reported similar findings across cortical areas (17,22,24); however, nodes within the cortical vsWM network have not been examined systematically.

Consequently, we sought to answer three questions regarding markers of glutamate and GABA neurotransmission in layer 3 across regions of the human cortical vsWM network. First, do gene products regulating key elements of glutamate and GABA transmission normally exhibit regional differences in expression in layer 3? Second, is the expression of these gene products altered in schizophrenia, and if so, are those alterations region specific or conserved across regions? Third, how do any disease effects on expression affect the normal regional patterns of glutamate and GABA transcript levels in the vsWM network?

To address these questions, we quantified the expression of key markers of glutamate and GABA neurotransmission in layer 3 from four regions of the vsWM network from 20 matched pairs of schizophrenia and unaffected comparison subjects. We selected the following functionally analogous markers of glutamate and GABA neurotransmission: 1) the enzymes that synthesize most cortical glutamate and GABA, glutaminase (GLS1) and the 67-kDa isoform of glutamic acid decarboxylase (GAD67), respectively; 2) vesicular glutamate transporter 1 (vGLUT1) and vesicular GABA transporter (vGAT), which package their respective neurotransmitters into presynaptic vesicles; 3) excitatory amino acid transporter 2 (EAAT2) and GABA transporter 1 (GAT1), involved in glutamate and GABA neurotransmitter reuptake, respectively; and 4) the obligatory *N*-methyl-D-aspartate receptor subunit (GRIN1), the calcium-impermeable alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunit (GRIA2), and the obligatory ionotropic $GABA_A$ receptor subunit $\gamma 2$ (GABRG2). Our experimental design that controlled for batch effects within subject pairs and across regions within subjects necessarily limited the number of transcripts that could be studied (see Methods and Materials, and Supplemental Methods), excluding the possibility of examining other glutamate or GABA receptor transcripts.

METHODS AND MATERIALS

Human Subjects

Human brain specimens ($N = 40$) were obtained during routine autopsies conducted at the Allegheny County Medical Examiner's Office (Pittsburgh, PA) following consent obtained from the next of kin. Consensus DSM-IV diagnoses were made by an independent committee of experienced research clinicians using structured interviews with family members and review of prior medical records (25). The absence of a psychiatric diagnosis was confirmed in unaffected comparison subjects using the same approach.

To control for experimental variance and reduce between-group biological variance, each subject with schizophrenia was matched with one unaffected comparison subject for sex and as closely as possible for age. Subject groups did not differ in mean age, pH, RNA integrity number (Agilent Bioanalyzer, Agilent Technologies, Santa Clara, CA), postmortem interval, or tissue storage time at −80°C (Table 1, Supplemental Table S1). All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research and Clinical Training Involving the Dead and Institutional Review Board for Biomedical Research.

Laser Microdissection Procedure

The right hemisphere of each brain was blocked coronally, immediately frozen, and stored at −80°C as previously described (25). Four regions (V1 [Brodmann area 17], V2 [Brodmann area 18], PPC [Brodmann area 7], DLPFC [Brodmann area 46]) were sampled based on their anatomic location and cytoarchitectonic features (Figure 1). Cryostat sections (12 μm) were cut; thaw-mounted onto glass polyethylene naphthalate membrane slides (Leica Microsystems, Bannockburn, IL), which were coded to blind subject number and diagnosis; dried; and stored at −80°C as previously described (26). On the day of microdissection, tissue sections were stained for Nissl substance with thionin, and layer 3 was identified based on its characteristic cytoarchitecture (Figure 1B) in portions of each section that were cut perpendicular to the pial surface. Strips (~10 million μm^2) containing layer 3 from each region were dissected (Supplemental Figure S1) using a Leica microdissection system (LMD 6500; 5× objective).

Quantitative Polymerase Chain Reaction Analyses

Samples from all four regions of both subjects within each pair were processed together throughout the study. For each sample, RNA was extracted and purified using the RNAeasy Plus Micro Kit (QIAGEN, Inc., Valencia, CA). Total RNA was converted to complementary DNA (cDNA) using the qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD). Forward and reverse primers were designed for each target messenger

Table 1. Summary of Demographic and Postmortem Characteristics of Human Subjects

Parameter	Unaffected Comparison Subjects	Subjects With Schizophrenia	Statistics
n	20	20	N/A
Sex, Male/Female	14/6	14/6	N/A
Race, White/Black	16/4	13/7	$\chi^2_1 = 1.1$; $p = .29$
Age, Years	47.2 ± 9.9	45.6 ± 9.5	$t_{38} = -0.5$; $p = .62$
PMI, Hours	15.4 ± 5.8	14.4 ± 6.2	$t_{38} = -0.5$; $p = .59$
Brain pH	6.7 ± 0.2	6.5 ± 0.3	$t_{38} = -1.6$, $p = .12$
RIN	8.3 ± 0.5	8.2 ± 0.6	$t_{38} = -0.6$, $p = .53$
Storage Time at −80°C, Months	134.7 ± 39.3	137.5 ± 49.3	$t_{38} = 0.2$; $p = .84$

Values are *n* or mean ± SD.

N/A, not applicable; PMI, postmortem interval; RIN, RNA integrity number.

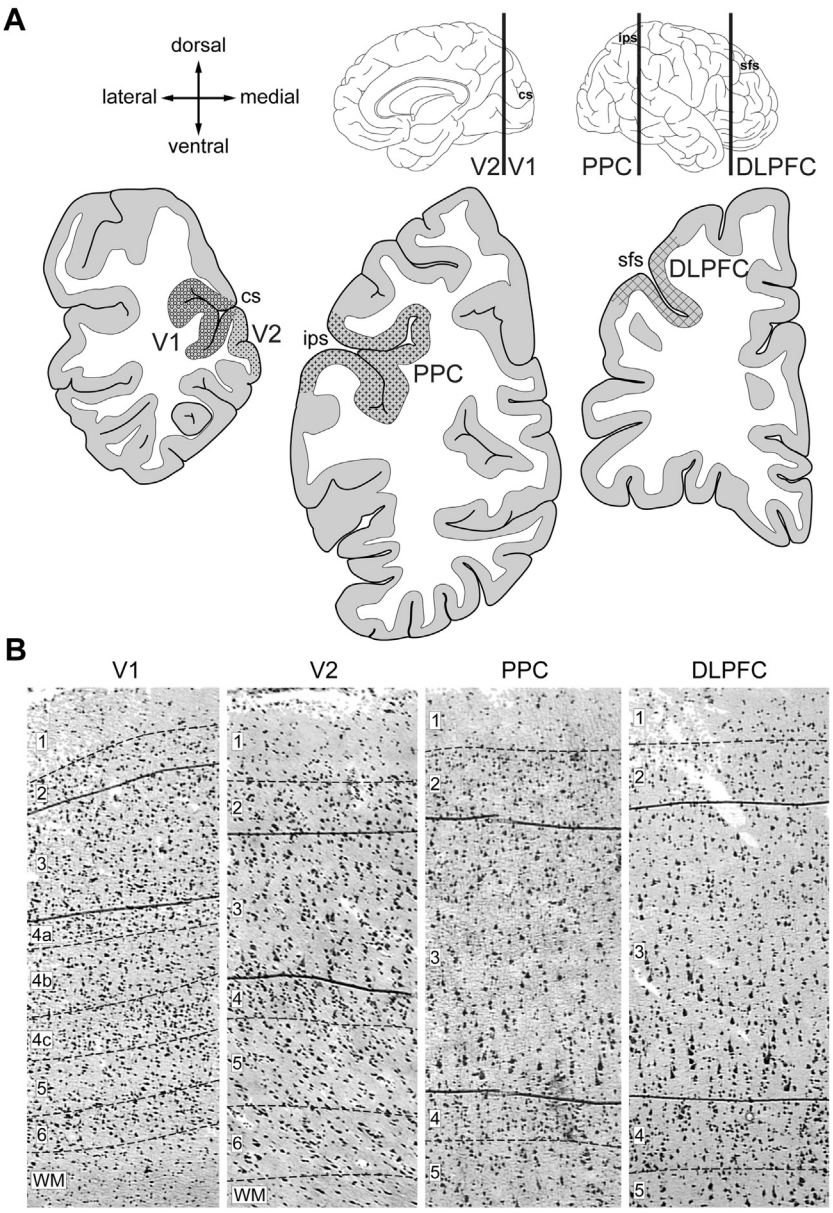

Figure 1. Sampling of cortical regions and layer 3. **(A)** Schematic drawings of the medial and lateral surfaces of the right hemisphere of the human brain (top). Vertical lines indicate the approximate locations of the coronal sections (bottom). Locations of cortical regions of the visuospatial working memory (WM) network selected for sampling are shaded. Arrows labeled dorsal, medial, ventral, and lateral refer to the coronal sections. **(B)** Representative Nissl-stained sections illustrating the laminar borders (dashed lines) and the borders as drawn of layer 3 for laser microdissection in each region. Numbers indicate cortical layers. cs, calcarine sulcus; DLPFC, dorsolateral prefrontal cortex; ips, intraparietal sulcus; PPC, posterior parietal cortex; sfs, superior frontal sulcus; V1, primary visual cortex; V2, association visual cortex.

RNA (mRNA) to generate polymerase chain reaction (PCR) amplicons of 85 to 120 bp (Supplemental Table S2). The specificity and efficiency of quantitative PCR amplification for each target mRNA was demonstrated by high amplification efficiency (>96%) across a wide range of cDNA dilutions, and the presence of singular products in dissociation curve analysis.

Transcript expression levels were quantified by quantitative PCR using Power SYBR green dye and ViiA 7 Real-Time PCR system (Life Technologies, Carlsbad, CA). For each subject pair, cDNA samples for all cortical regions were processed together on the same 384-well quantitative PCR plate with four replicates per primer set. To avoid batch effects, all transcripts from all regions of both subjects in a pair were run on the same plate, limiting the number of transcripts that could be studied. Splice variants exist for each targeted transcript, but the primer sets amplified all variants for all transcripts except EAAT2 and GRIA2, where the synaptically enriched splice variants were amplified (Supplemental Table S2).

Using an established method (27), the comparative cycle threshold approach was used to normalize transcript levels to the geometric mean of the internal reference genes (β-actin and cyclophilin A), which were previously reported to have stable expression levels across multiple cortical regions and diagnoses (17,25,28–30), and were not significantly different across region or diagnosis in this cohort (Supplemental Methods). Because the difference in cycle threshold between

the cycle thresholds of each target transcript and the mean of the reference genes represents the log2-transformed expression ratio of each target transcript to the reference genes mean, the relative expression ratio of each target transcript was determined as $2^{-\text{difference in cycle threshold}}$ (17,25).

Statistical Analyses

To compare transcript levels across regions in unaffected comparison subjects, a mixed model treating observations from the four regions for each subject as repeated measurements was performed for each transcript. The model included transcript as the dependent variable; region as a fixed effect; and age, sex, brain pH, RNA integrity number, postmortem interval, and tissue storage time as covariates. *F* tests were used to assess the overall region effect, followed by post hoc pairwise comparisons between regions, using Tukey's method to control the overall type I error.

To determine if the expression of any transcripts was altered in schizophrenia, a mixed model was performed across all subjects for each individual transcript, where fixed effects included diagnosis, region, and diagnosis-by-region interaction, while controlling for the covariates listed above. The diagnosis and diagnosis-by-region interaction effects were tested using *F* tests. The potential confounding effect of antipsychotic medications, nicotine, or other substances of abuse, suicide, and other factors frequently comorbid with schizophrenia was also examined (Supplemental Table S3).

Composite scores of the glutamate and GABA measures were computed by summing the normalized (*Z* score) expression levels for all glutamate and GABA transcripts, respectively (Supplemental Methods). For each composite measure, a mixed model was performed across all subjects, where fixed effects included diagnosis, region, and diagnosis-by-region interaction, while controlling for the covariates listed above. The diagnosis and diagnosis-by-region interaction effects were tested using *F* tests.

All analyses were conducted on log-transformed data to stabilize the variance. Adjustments for multiple comparisons were made using the Benjamini-Hochberg method to control for the false discovery rate. For the main analyses, the uncorrected *p* value is reported in the results. Corrected *p* values for the comorbidity and main analyses are reported in Supplemental Tables S3 and S4. Models used SAS version 9.3 (SAS Institute, Cary, NC) to implement PROC MIXED using the restricted maximum likelihood method such that the default ("containment") method was used to compute the denominator degrees of freedom.

RESULTS

Glutamate and GABA Transcript Levels in the vsWM Network of Comparison Subjects

Three glutamate transcripts (Figure 2B, C, E; Supplemental Table S4), vGLUT1 ($F_{3,56}$ = 57.1, $p < .0001$), EAAT2 ($F_{3,56}$ = 19.2, $p < .0001$), and GRIA2 ($F_{3,56}$ = 59.3, $p < .0001$), showed similar patterns of increasing levels of expression from the caudal to rostral regions (Figure 1A) of the vsWM network. Post hoc analyses confirmed lower expression in the V1 and V2 compared with the PPC and DLPFC for each of these transcripts. For vGLUT1 and GRIA2, expression levels were also significantly lower in the PPC than in the DLPFC. In contrast, GLS1 and GRIN1 mRNA levels did not differ across the cortical regions studied (Figure 2A, D).

Three GABA transcripts (Figure 2F, G, I; Supplemental Table S4), GAD67 ($F_{3,56}$ = 4.52, $p < .007$), vGAT ($F_{3,56}$ = 11.8, $p < .0001$), and GABRG2 ($F_{3,56}$ = 34.1, $p < .0001$), shared a similar pattern of decreasing levels of expression from the caudal to rostral regions of the vsWM network. Post hoc analyses confirmed higher expression in the V1 than in the DLPFC for each transcript. In contrast, GAT1 mRNA levels did not differ across the cortical regions studied (Figure 2H).

The consistency of expression differences between the V1 and DLPFC was supported by findings within individual subjects. For glutamate transcripts, vGLUT1 and GRIA2 levels were lower in the V1 than in the DLPFC in all 20 subjects, and EAAT2 was lower in the V1 than in the DLPFC in 17 of 20 subjects (Supplemental Figure S2B, C, E). For GABA transcripts, GABRG2 levels were higher in the V1 than in the DLPFC in all 20 subjects, and vGAT and GAD67 were higher in the V1 than in the DLPFC in 17 and 16 of 20 subjects, respectively (Supplemental Figure S2F, G, I).

Effect of Schizophrenia on Expression of Glutamate and GABA Transcripts in the vsWM Network

Next, we studied whether schizophrenia was associated with alterations in layer 3 glutamate and GABA transcript levels and whether any such alterations were conserved across regions (Figure 3). For glutamate transcripts, vGLUT1 mRNA expression significantly differed by diagnosis ($F_{1,114}$ = 9.3, $p < .003$) and region ($F_{3,114}$ = 56.1, $p < .0001$), but the interaction term was not significant. Post hoc analyses showed that mean transcript levels for vGLUT1 (Figure 3B) were significantly lower in schizophrenia in all regions studied (V1: −18%, $p < .005$, corrected p = .03; V2: −14%, $p < .03$, corrected p = .12; PPC: −14%, $p < .03$, corrected p = .12; and DLPFC: −22%, p = .005, corrected p = .04). Expression of EAAT2 mRNA showed significant effects of diagnosis ($F_{1,114}$ = 5.6, $p < .02$) and region ($F_{3,114}$ = 4.5, $p < .005$), as well as a significant region-by-diagnosis interaction ($F_{3,114}$ = 16.0, $p < .0001$). Mean EAAT2 mRNA levels (Figure 3C) were higher in the V1 (+286%, $p < .0005$, corrected p = .003) and V2 (+258%, $p < .0001$, corrected p = .004) in schizophrenia (also see Supplemental Figure S3), and in the PPC (+61%) and DLPFC (+7%), although the disease effect in the latter two regions did not achieve statistical significance. For GLS1, GRIN1, and GRIA2 mRNAs (Figure 3A, D, E) neither the effect of diagnosis nor the region-by-diagnosis interaction was significant; however, the effects of region on mRNA levels of GRIA2 ($F_{3,114}$ = 93.3, $p < .0001$, corrected $p < .001$) and GRIN1 ($F_{3,114}$ = 9.9, $p < .0001$, corrected $p < .001$), but not GLS1, were significant. GRIN1 transcript expression (Figure 3D) was higher in the V1 (+28%, p = .02, corrected p = .11) and V2 (+18%, p = .10, corrected p = .33) in schizophrenia, although the latter finding in V2 did not achieve statistical significance.

For each GABA transcript, region had a significant effect on mRNA levels in layer 3 (all $F_{1,114} > 8.0$, all $p < .0001$), but there was no effect of diagnosis for any transcript (all $F_{1,114} < 2.7$, all $p > .10$). Region-by-diagnosis interaction was significant for vGAT ($F_{3,114}$ = 2.8, $p < .05$, corrected p = .08) and GAT1

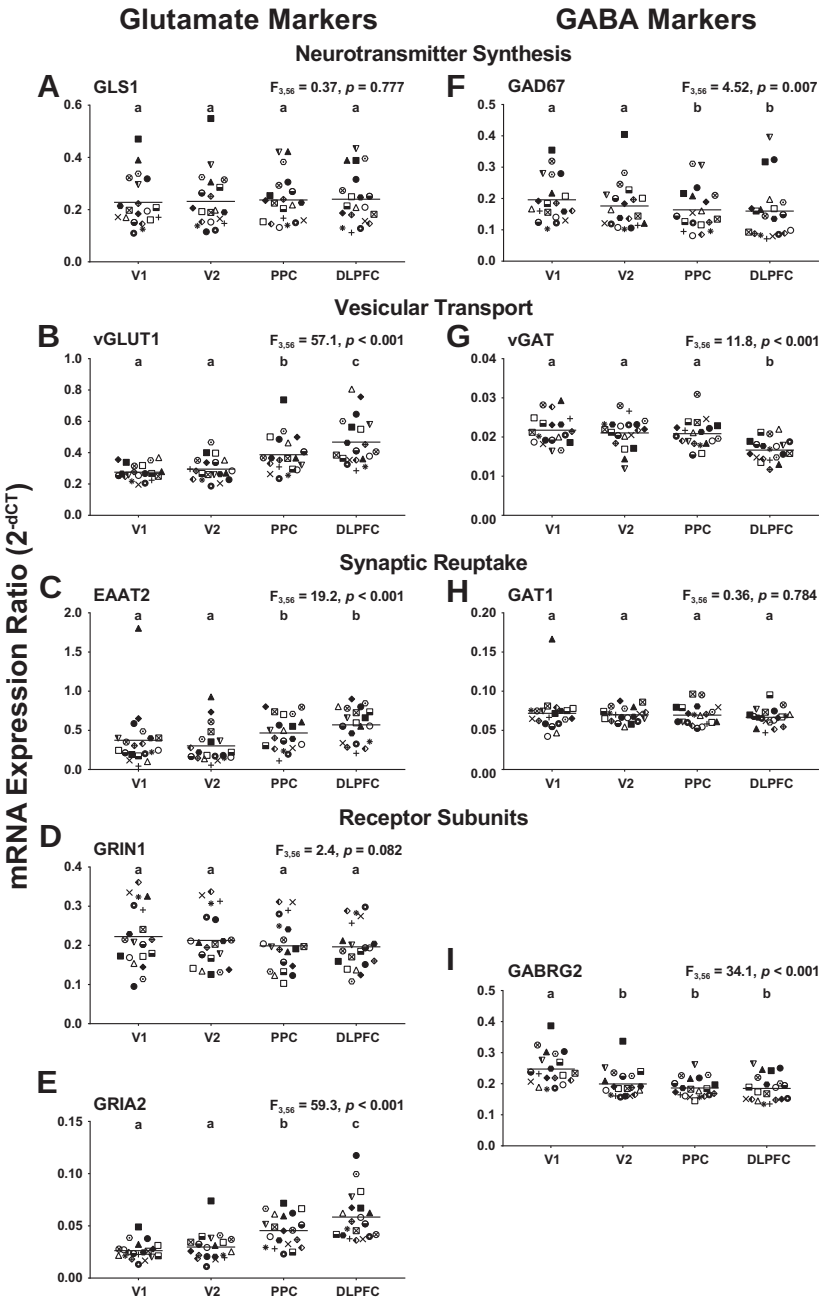

Figure 2. Glutamate and gamma-aminobutyric acid (GABA) transcript levels in layer 3 across cortical regions of the visuospatial working memory (vsWM) network in unaffected comparison subjects. For each panel **(A–I)**, target transcript name is at the top center and analysis of covariance results for the effect of region at the top right. Individual subjects are shown by the same symbol in all graphs. Horizontal bars represent group means. Regions within each graph that do not share the same letter are significantly different ($p < .05$). DLPFC, dorsolateral prefrontal cortex; EAAT2, excitatory amino acid transporter 2; GABRG2, gamma-aminobutyric acid type A receptor subunit γ2; GAD67, 67 kDa isoform of glutamic acid decarboxylase; GAT1, gamma-aminobutyric acid membrane transporter 1; GLS1, glutaminase; GRIA2, glutamatergic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunit; GRIN1, *N*-methyl-D-aspartate receptor subunit; mRNA, messenger RNA; PPC, posterior parietal cortex; V1, primary visual cortex; V2, association visual cortex; vGAT, vesicular gamma-aminobutyric acid transporter; vGLUT1, vesicular glutamate transporter 1.

($F_{3,114}$ = 5.9, $p < .0001$, corrected $p = .003$), but not for GAD67 or GABRG2. Post hoc analyses revealed that vGAT levels (Figure 3G) were significantly lower in the PPC (−19%, $p < .005$, corrected $p = .03$) in schizophrenia, but were not different in the V1, V2, or DLPFC. GAT1 mRNA levels (Figure 3F) were higher in the V1 (+57%, $p = .07$, corrected $p = .29$) and GAD67 mRNA levels were lower in the DLPFC (−17%, $p = .10$, corrected $p = .33$) in schizophrenia, but these differences did not achieve statistical significance.

None of the schizophrenia-associated comorbid factors examined (death by suicide, or the use of antipsychotics, antidepressants, anticonvulsants/benzodiazepines, or nicotine at the time of death) had a significant effect on the levels of any transcript in any region.

Composite Measures of Glutamate and GABA Transcripts Across Nodes of the vsWM Network: Effects of Schizophrenia

As described in Methods and Materials, we computed composite glutamate and GABA measures from the normalized expression levels of the relevant transcripts. As expected, in

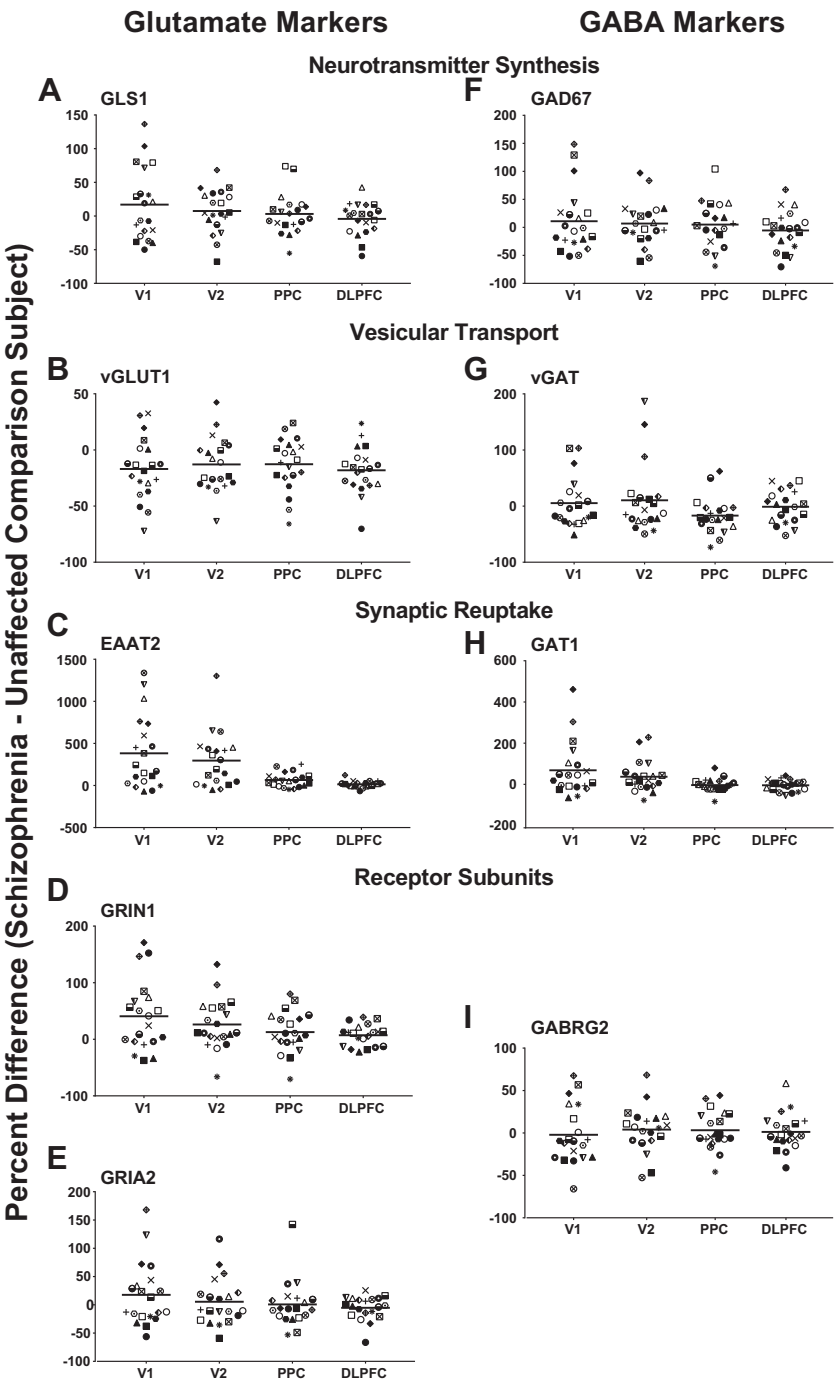

Figure 3. Effect of schizophrenia on glutamate and gamma-aminobutyric acid (GABA) transcript levels across cortical regions of the visuospatial working memory (vsWM) network. For each panel **(A–I)**, target transcript name is at the top center. Individual subjects are shown by the same symbol in all graphs. Horizontal bars represent mean percent difference (schizophrenia – unaffected comparison subject). DLPFC, dorsolateral prefrontal cortex; EAAT2, excitatory amino acid transporter 2; GABRG2, gamma-aminobutyric acid type A receptor subunit $\gamma 2$; GAD67, 67 kDa isoform of glutamic acid decarboxylase; GAT1, gamma-aminobutyric acid membrane transporter 1; GLS1, glutaminase; GRIA2, glutamatergic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunit; GRIN1, *N*-methyl-D-aspartate receptor subunit; mRNA, messenger RNA; PPC, posterior parietal cortex; V1, primary visual cortex; V2, association visual cortex; vGAT, vesicular gamma-aminobutyric acid transporter; vGLUT1, vesicular glutamate transporter 1.

unaffected comparison subjects the composite glutamate ($F_{3,114}$ = 14.5, $p < .0001$) and GABA ($F_{3,114}$ = 5.4, $p < .002$) measures confirmed significant regional differences in expression (Figure 4). Like most individual transcripts, the glutamate composite measure showed increasing expression and the GABA composite measure showed decreasing expression from the caudal to rostral regions of the vsWM network. However, in the schizophrenia subjects this caudal-to-rostral gradient was lost for the glutamate measure ($F_{3,114}$ = 1.3, p = .28), but it was more highly significant for the GABA measure ($F_{3,114}$ = 15.8, $p < .0001$). For example, the difference between the V1 and DLPFC in the composite glutamate measure was much greater in unaffected comparison subjects than in schizophrenia subjects, whereas for the GABA measure this difference was greater in

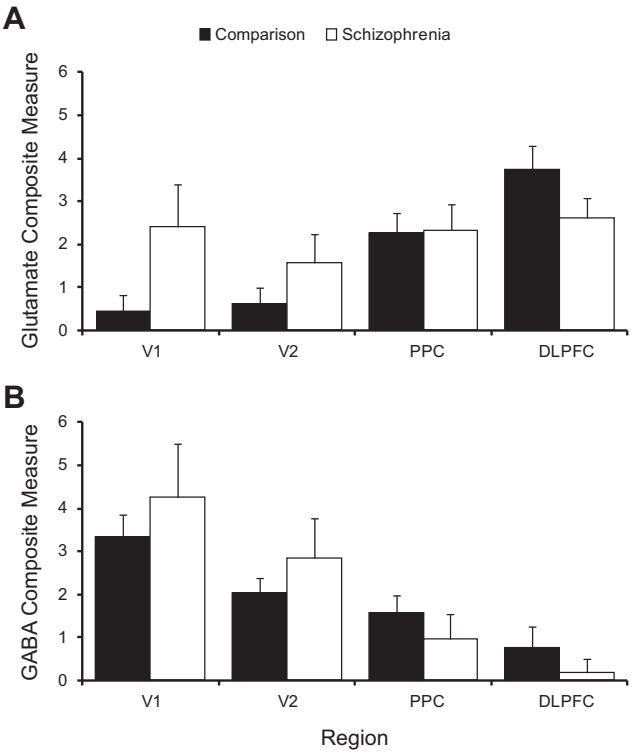

Figure 4. Effect of schizophrenia on composite measures of glutamate and gamma-aminobutyric acid (GABA) transcripts in the visuospatial working memory network. In unaffected comparison subjects, the composite **(A)** glutamate ($F_{3,114}$ = 14.5, $p < .001$) and **(B)** GABA ($F_{3,114}$ = 5.4, p = .002) measures showed significant, and opposite, caudal-to-rostral gradients. Note that in the schizophrenia subjects, this gradient was lost for the glutamate measure ($F_{3,114}$ = 1.3, p = .28), but was more highly significant for the GABA measure ($F_{3,114}$ = 15.8, $p < .001$). Accordingly, the region-by-diagnosis interaction was significant for the glutamate ($F_{3,114}$ = 5.1, p = .002), but not for the GABA ($F_{3,114}$ = 1.7, p = .18), composite measure. DLPFC, dorsolateral prefrontal cortex; PPC, posterior parietal cortex; V1, primary visual cortex; V2, association visual cortex.

schizophrenia than in unaffected comparison subjects (Figure 4). The diagnosis-by-region interaction was significant for glutamate ($F_{3,114}$ = 5.1, p = .002), but not for GABA ($F_{3,114}$ = 1.7, p = .18), and the 3-way interaction of diagnosis, region, and neurotransmitter composite type (glutamate or GABA) was not significant ($F_{3,265}$ = 0.6, p = .61). An additional analysis excluding EAAT2 from the glutamate composite measure showed a similar diagnosis-by-region interaction with reduced statistical significance ($F_{3,114}$ = 2.7, p = .05).

DISCUSSION

In this study, we found that most glutamate and GABA transcripts examined exhibited opposite caudal-to-rostral gradients of expression across nodes of the cortical vsWM network, with glutamate transcript levels increasing and GABA transcript levels decreasing from the caudal (V1) to rostral (DLPFC) regions. In schizophrenia subjects, the expression levels of many transcripts were altered, but the disease effect was not conserved across cortical regions. This differential effect of schizophrenia across regions altered the normal caudal-to-rostral gradients of expression in the vsWM network such that the glutamate marker gradient was blunted and the GABA marker gradient was enhanced in the illness.

Altered Caudal-to-Rostral Gradients of Glutamate and GABA Neurotransmission Markers in Schizophrenia

Overall, schizophrenia was associated with region-specific alterations in the expression of glutamate and GABA transcripts in layer 3 of the cortical vsWM network such that both glutamate and GABA transcripts were upregulated in the V1 and V2, downregulated in the DLPFC, and modestly downregulated or unchanged in the PPC (Figure 4). In combination, the region-specific changes in expression of glutamate and GABA transcripts in schizophrenia resulted in disease-related shifts in the normal caudal-to-rostral patterns of gene expression such that the glutamate marker gradient was blunted and the GABA marker gradient was enhanced in the illness. Thus, although speculative, in schizophrenia deficits in both glutamate and GABA neurotransmission in the DLPFC might impair the ability of layer 3 microcircuitry to increase the power of gamma oscillations required for vsWM function (31), whereas elevated levels of both glutamate and GABA neurotransmission in layer 3 of the V1 might contribute to the elevated levels of visual gamma power reported in psychosis (32). Such disease-related shifts in regional patterns of gene expression might also provide a molecular basis for the observation that the normal caudal-to-rostral gradient in the natural frequency of cortical oscillations is disrupted in schizophrenia (33).

The shifts in the caudal-to-rostral gradients of glutamate and GABA markers reflect both conserved and region-specific effects of the illness on the expression of individual transcripts. For example, vGLUT1 mRNA expression was lower in schizophrenia in each of the four cortical regions examined, suggesting a deficit in the amount of glutamate available for synaptic release (34) across the cortical vsWM network, which might contribute to the reduced activation of this network when individuals with schizophrenia are challenged with demanding WM tasks (35). However, depending on task conditions and possibly stage of illness, increased activation of the vsWM network has also been reported (36). It is important to note that alterations in vGLUT1 mRNA are likely to affect the vGLUT1 protein in the axon terminals of both the local collaterals and long-range projections of layer 3 pyramidal neurons.

In contrast to the conserved regional alterations in vGLUT1 mRNA, mean GAD67 mRNA levels in layer 3 were not altered in the V1, but levels were 17% lower in the DLPFC from subjects with schizophrenia. Although this difference did not reach statistical significance, the magnitude of the difference is quite similar to multiple prior studies, which reported that mean GAD67 mRNA levels in total DLPFC gray matter were 12% to 36% lower in schizophrenia (23). Indeed, the largest single study (N = 62 subject pairs) reported a 14% decrease in mean GAD67 mRNA levels in DLPFC gray matter (30). The absence of a difference of GAD67 mRNA levels in the V1 appears to contrast with a prior study (17). However, it is important to

note that the latter study specifically selected schizophrenia subjects (n = 9 pairs) with the largest deficits in GAD67 mRNA expression in the DLPFC and included all six layers of the V1, whereas the present study examined only layer 3 in the V1 from subject pairs selected based on the highest quality of RNA independent of any prior knowledge of DLPFC GAD67 levels. In addition, our finding of no alterations in vGAT mRNA levels in the DLPFC is consistent with the prior findings of another group (29), and perhaps not materially different from our prior report of a 7% decrease (p = .046) in schizophrenia in total DLPFC gray matter (37). Together, these comparisons suggest that disease-related transcript alterations might be selective for specific layers or cell types in certain cortical regions, an interpretation consistent with prior reports of schizophrenia-associated transcript alterations selective for pyramidal neurons (26) or subpopulations of GABAergic neurons (28).

Several factors need to be considered in interpreting the regional patterns of glutamate and GABA transcript levels. First, the V1 has a much greater cell packing density relative to other cortical regions (38). In addition, in most regions of the primate neocortex, the ratio of interneurons to principal cells is 1:4, whereas in the V1 the ratio is 1:3 (38). Thus, although we collected the same volume of layer 3 tissue from each region of every subject, the V1 samples likely contained more neurons and a greater proportion of GABA neurons. Consistent with these findings in the unaffected comparison subjects, GABA transcript levels were higher in the V1 than in other regions; however, glutamate transcript levels were lower (Figure 4). Furthermore, levels of glutamate and GABA transcripts were lower and higher, respectively, in V2 than in the PPC and DLPFC, even though cell packing densities in layer 3 appear to be similar across these three regions (38). Thus, our findings do not appear to be confounded by regional differences in cytoarchitecture.

Second, findings from transcriptome studies in monkeys and humans demonstrated that multiple gene groups show a caudal-to-rostral increase or decrease in expression levels (12,14,15,39). Consistent with our results, these studies of tissue homogenates containing all cortical layers found the highest levels of vGLUT1 and GRIA2 mRNAs in the frontal lobe and the highest levels of GAD67 mRNA in the occipital lobe (12,13). Other markers of glutamate neurotransmission (e.g., Ca^{2+}/calmodulin-dependent kinase [CAMK2B] and certain alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and *N*-methyl-D-aspartate receptor subunits) had the highest levels of expression in the frontal cortex, whereas certain markers of GABA neurotransmission had the highest expression levels in the occipital lobe (13). These findings support the concept that caudal-to-rostral gradients of expression across cortical regions are present for multiple gene groups and are in the opposite direction for key gene products regulating glutamate and GABA neurotransmission. Importantly, the glutamate and GABA composite measures capture only the transcripts assessed in the present study and thus do not serve as measures of the entire glutamate or GABA neurotransmitter systems.

Third, like all postmortem studies, we cannot definitively exclude the potential influence of other factors comorbid with schizophrenia. Statistical analyses suggest that the factors investigated did not significantly affect transcript expression levels in this cohort (Supplemental Table S3). Furthermore, the mean body mass index at time of death in the schizophrenia subjects was 29 kg/m^2, which is at the top of the overweight range, suggesting that malnutrition is unlikely to account for altered gene expression in schizophrenia.

Functional Implications

Visuospatial information is conveyed in a hierarchical fashion from the caudal (V1) to rostral (DLPFC) cortices (9,40). Given their larger dendritic arbors and greater densities of dendritic spines (18,19,41), layer 3 pyramidal neurons in the DLPFC can receive more excitatory inputs than those in the V1, which may support a more extensive level of information integration in higher-order association areas of the vsWM network. Consistent with this idea, computational models support regional differences in the strength of local recurrent excitation, with greater strength in association than primary sensory cortices (42–44). Accordingly, our findings in unaffected comparison subjects suggest that a pattern of low glutamate/high GABA transcript expression may contribute to relatively lower local neural network activity in layer 3 of the V1 compared with the high glutamate/low GABA transcript expression pattern in layer 3 of the DLPFC. Functionally, for example, high glutamate/low GABA transcripts in DLPFC might reflect an increase in sustained recurrent excitation that allows for the integration of information and enhanced signal-to-noise ratio required for WM (43,45), whereas low glutamate/high GABA transcript ratio in the V1 might reflect a greater need for rapidly detecting and faithfully tracking dynamic stimuli in early sensory processing (43,46). However, this interpretation is limited by the difficulty of determining the functional consequences of alterations in transcript levels. For example, because GRIA2, GRIN1, and GABRAG2 are expressed on pyramidal cells and GABAergic interneurons (47), the cellular source of the expression difference would have a substantial effect on its physiological consequences. Cell type–specific and protein-level studies are needed to address these questions.

Comparisons between subject groups suggest that the set point for excitatory/inhibitory balance in layer 3 circuitry is lower in the DLPFC and higher in the V1 in schizophrenia. These regional differences might help account for findings that excitatory/inhibitory balance seems to be maintained by either comparable increases or decreases in both excitation and inhibition in different cortical networks in schizophrenia (31,48). However, in vivo studies of cortical network activity and of synaptic levels of glutamate and GABA across cortical regions are needed to determine if excitatory/inhibitory balance in schizophrenia is differentially altered as a function of cortical region or stage of the illness (49).

Finally, the precise balance of activity between glutamatergic pyramidal neurons and GABAergic interneurons in layer 3 is thought to be critical for optimal vsWM function (50). Our findings suggest that this balance might be altered in a region-specific manner in schizophrenia. Thus, disruptions in each region of the network could provide multiple, and potentially compounding, paths to vsWM dysfunction in the illness.

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