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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.