

Expression of Transcripts Selective for GABA Neuron Subpopulations across the Cortical Visuospatial Working Memory Network in the Healthy State and Schizophrenia

メタデータ	言語: eng 出版者: 公開日: 2019-08-30 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	https://doi.org/10.24517/00053879

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Journal:	<i>Cerebral Cortex</i>
Manuscript ID	CerCor-2018-00325.R1
Manuscript Type:	Original Articles
Date Submitted by the Author:	01-Jul-2018
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Keywords:	postmortem, parvalbumin, somatostatin, vasoactive intestinal peptide, calretinin

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7 Visuospatial Working Memory Network [in the Healthy State](#) and Schizophrenia
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11 Running title: GABA neuron transcripts in a cortical network in schizophrenia
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Abstract

Visuospatial working memory (WM), which is impaired in schizophrenia, depends on a distributed network including visual, posterior parietal and dorsolateral prefrontal cortical regions. Within each region, information processing is differentially regulated by subsets of GABA neurons that express parvalbumin (PV), somatostatin (SST) or vasoactive intestinal peptide (VIP). In schizophrenia, WM impairments have been associated with alterations of PV and SST neurons in the dorsolateral prefrontal cortex. Here, we quantified transcripts selectively expressed in GABA neuron subsets across four cortical regions in the WM network from comparison and schizophrenia subjects. In comparison subjects, PV mRNA levels declined and SST mRNA levels increased from posterior-to-anterior regions, whereas VIP mRNA levels were comparable across regions except for the primary visual cortex (V1). In schizophrenia subjects, each transcript in PV and SST neurons exhibited similar alterations across all regions, whereas transcripts in VIP neurons were unaltered in any region except for V1. These findings suggest that the contribution of each GABA neuron subset to inhibitory regulation of local circuitry normally differs across cortical regions of the visuospatial WM network, and that in schizophrenia alterations of PV and SST neurons are a shared feature across these regions, whereas VIP neurons are affected only in V1.

Keywords: postmortem, parvalbumin, somatostatin, vasoactive intestinal peptide, calretinin

Introduction

Working memory (WM), the ability to transiently maintain and manipulate information to guide thought or behavior, is mediated by neural networks distributed across multiple cortical regions (Christophel et al. 2017). For example, the visuospatial WM network includes primary (V1) and association (V2) visual regions in the occipital cortex that encode low-level details of visual inputs, as well as higher-order association regions, such as the posterior parietal cortex (PPC) and the dorsolateral prefrontal cortex (DLPFC) that process complex information (Eriksson et al. 2015; Christophel et al. 2017). Excitatory pyramidal neurons furnish the connections linking the cortical regions of the WM network, and inhibitory neurons that utilize γ -aminobutyric acid (GABA) shape pyramidal neuron firing during WM tasks (Rao et al. 2000; Constantinidis et al. 2002; Kim et al. 2016).

Cortical GABA neurons are classified into three major, non-overlapping subsets based on the expression of parvalbumin (PV), somatostatin (SST) or vasoactive intestinal peptide (VIP) (Pfeffer et al. 2013; Lake et al. 2016; Tremblay et al. 2016). These subsets appear to have distinct roles in cortical information processing. For example, PV neurons, via their perisomatic innervation of pyramidal neurons, regulate coincident input detection, response gain and synchronization of nearby pyramidal neurons (Tremblay et al. 2016). In contrast, SST neurons, via their inputs to pyramidal neuron distal dendrites, modulate dendritic integration and contribute to processing and encoding of specific information by pyramidal neurons (Kim et al. 2016; Tremblay et al. 2016; Kamigaki and Dan 2017). Finally, VIP neurons, by virtue of their inhibitory synapses on SST neurons and to a lesser extent on PV neurons, provide disinhibition of pyramidal neurons (Pfeffer et al. 2013; Pi et al. 2013; Tremblay et al. 2016). In non-human primates, the neuronal density of each GABA neuron subset differs among cortical regions and these differences are thought to reflect regionally-specific requirements of each subset for local information processing (Hayes et al. 1991; Kondo et al. 1994; Conde et al. 1996; Defelipe et al.

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3 1999).

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5 In subjects with schizophrenia, WM impairments, a core cognitive deficit in the illness (Silver
6 et al. 2003; Barch and Ceaser 2012), have been attributed, at least in part, to altered inhibition in the
7 DLPFC (Marin 2012), which appears to involve PV and SST neurons. First, multiple postmortem
8 studies reported reduced levels of PV and SST mRNAs in the DLPFC of subjects with schizophrenia
9 (Hashimoto et al. 2003; Morris et al. 2008; Mellios et al. 2009; Fung et al. 2010; Fung et al. 2014;
10 Guillozet-Bongaarts et al. 2014; Chung et al. 2016b). Second, reduced transcript levels were also
11 reported for KCNS3, a potassium channel subunit that is highly selective to PV neurons in the human
12 neocortex (Georgiev et al. 2014), and LIM homeobox 6 (LHX6) transcription factor (Volk et al. 2012a),
13 which is selectively expressed in both PV and SST neurons in the human neocortex (Georgiev et al.
14 2012). Third, μ -opioid receptor (MOR), which was primarily detected in most PV neurons and some
15 SST neurons in the rodent hippocampus (Drake and Milner 2002; Stumm et al. 2004), exhibited elevated
16 transcript levels in the DLPFC of schizophrenia subjects (Volk et al. 2012b). Finally, levels of GAD67,
17 an enzyme for GABA synthesis, was reduced in PV neurons (Hashimoto et al. 2003; Curley et al. 2011).
18 In contrast, transcripts selective for VIP neurons, including VIP and calretinin (CR) (Kubota et al. 1994;
19 Gabbott and Bacon 1997; Lake et al. 2016), are relatively unaltered in the DLPFC of schizophrenia
20 subjects (Hashimoto et al. 2003; Fung et al. 2010; Fung et al. 2014; Guillozet-Bongaarts et al. 2014;
21 Chung et al. 2016b).

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23 Given the functional differences among cortical regions of the visuospatial WM network, the
24 distinct roles of the GABA neuron subsets in cortical processing, and the regionally different densities of
25 each GABA neuron subset, we asked how the contribution of each GABA neuron subset to local
26 circuitry differs across cortical regions of the WM network in unaffected comparison subjects.
27 Furthermore, in schizophrenia, the selective alterations in PV and SST neurons in the DLPFC raise the
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3 question of whether the neural substrate for visuospatial WM dysfunction involves shared or distinct
4 regional alterations in GABA neuron subsets. To answer these questions, we quantified transcript levels
5 for markers of each GABA neuron subset across four cortical regions (V1, V2, PPC and DLPFC) of the
6 visuospatial WM network from 20 matched pairs of unaffected comparison and schizophrenia subjects.
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For Peer Review

Materials and Methods

Human subjects

Brain specimens from unaffected comparison (n=20) and schizophrenia subjects (n=20) were obtained during autopsies conducted at the Allegheny County Medical Examiner's Office, Pittsburgh, PA after consent was obtained from the next-of-kin. An independent committee of experienced research clinicians verified the absence of any psychiatric diagnosis, or made consensus DSM-IV diagnoses, for each subject on the basis of structured diagnostic interviews with family members and medical records. To reduce biological and control for experimental variances, each schizophrenia subject was matched with an unaffected comparison subject for sex and, as closely as possible for age and postmortem interval, and tissue samples from both members of each pair were always processed together. The mean age, postmortem interval, RNA integrity number (Bioanalyzer, Agilent Technologies, Santa Clara, CA), brain pH and tissue storage time did not differ between subject groups (Table 1, Supplementary Table 1). 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, as well as by the Ethics Committee of Kanazawa University Graduate School of Medical Sciences.

Tissue preparation

The right hemisphere of each brain was blocked coronally, immediately frozen, and stored at -80°C . Four cortical regions (V1, V2, PPC and DLPFC) were identified in Nissl-stained sections from the immersion-fixed left hemisphere based on cytoarchitectonic features and then sampled from the homotopic region in the fresh-frozen right hemisphere (Figure 1); the identity of each sampled region was confirmed by Nissl-staining of adjacent sections (Hoftman et al. 2018). The gray matter (GM) of

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3 each region was dissected from 40- μ m-thick cryostat sections in areas where the tissue was cut
4 perpendicular to the pial surface and immediately homogenized in TRIzol reagent (Invitrogen, Carlsbad,
5 CA).
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12 **Real-time PCR analyses**

14 Total RNA was isolated from the homogenate samples of each cortical region using RNeasy lipid tissue
15 mini kit (Qiagen, Hilden, Germany) and converted into cDNA with High Capacity RNA-to-cDNA Kit
16 (Applied Biosystems, Foster City, CA). The fragments of each transcript were amplified using Power
17 SYBR Green PCR Master Mix and StepOnePlus real-time PCR system (Applied Biosystems). All
18 primer sets (Supplementary Table 2) amplified specific, single products in dissociation curve analyses
19 and had amplification efficiency $\geq 96\%$ in standard curve analyses. All amplified fragments had expected
20 sizes. For each subject pair and each cortical region, eight target transcripts selectively expressed in
21 GABA neuron subsets and two internal control transcripts (beta-actin and cyclophilin-A) were amplified
22 in one qPCR run as quadruplicates. Cycle threshold (CT) of each transcript was determined as the
23 average across quadruplicates.
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37 As the difference in cycle threshold (dCT) between the CT of each target transcript and the
38 mean CT of beta-actin and cyclophilin-A represents \log_2 -transformed expression ratio of the target
39 transcript to the geometric mean of the two control transcripts, the expression levels of each target
40 transcript were determined as 2^{-dCT} .
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47 For each target transcript in each region, the difference in expression levels between
48 comparison and schizophrenia subjects in each pair was determined as $-ddCt$ [$-(dCT$ for the
49 schizophrenia subject $- dCT$ for the comparison subject)], and the within-pair expression ratio of
50 schizophrenia and comparison subjects was determined as 2^{-ddCT} .
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Statistical analyses

Mixed models treating observations from the four regions for each subject as repeated measurements were used to account for the within subject correlation among four regions, and characterize the within and between subject variability. To compare the target transcript levels across the four regions in comparison subjects, the mixed model included transcript level as the dependent variable, region as the fixed effect, and sex, age, PMI, storage time, RIN and brain pH as covariates. F-tests were used to assess the overall regional effect, followed by post-hoc pairwise comparisons. To assess if the expression of these transcripts was altered in schizophrenia, the mixed model included transcript level as the dependent variable, diagnosis, region and diagnosis-by-region interaction as the fixed effects, and the same set of covariates. The diagnosis effect and diagnosis-by-region interaction effect were tested using F-tests, followed by post-hoc comparisons in each region. The denominator degrees of freedom (57 for the evaluation of regional effects on transcript levels in comparison subjects and 114 for the assessment of the effect of schizophrenia on transcript levels), computed using SAS PROC MIXED (“containment”), correspond to effective sample sizes that are larger than the numbers of subjects (20 and 40, respectively) but smaller than the numbers of samples (80 and 160, respectively) (Jiang 2007). The Holm simultaneous inference procedure (Holm 1979) was used to correct for multiple comparisons across eight transcripts (Volk et al. 2000; Morris et al. 2008) and reported P values for each transcript have been adjusted to correspond to the family-wise error rate of 0.05.

Results

Expression of GABA neuron transcripts in unaffected subjects

Transcripts selectively expressed in the three GABA neuron subsets exhibited distinctive expression patterns across the four regions of the visuospatial WM network. PV mRNA levels declined steeply from the posterior to anterior regions ($F_{3,57}=244$, $P<0.001$) and differed significantly among all four regions (Figure 2A). Similarly, KCNS3 mRNA, which is selectively expressed in PV neurons in the human cortex (Georgiev et al. 2012), showed a posterior-to-anterior decline ($F_{3,57}=30.8$, $P<0.001$), although the differences in KCNS3 mRNA levels across regions were smaller and not significant between V1 and V2. KCNS3 mRNA levels were significantly higher in V1 and V2 than in PPC, and significantly lower in DLPFC than in PPC (Figure 2B).

In contrast to PV neuron markers, SST mRNA levels increased from the posterior to anterior regions ($F_{3,57}=68.2$, $P<0.001$) and differed significantly among all four regions (Figure 2C). In a manner remarkably similar to SST mRNA, levels of MOR mRNA, reported to be present in both PV and SST neurons (Drake and Milner 2002; Stumm et al. 2004), increased from the posterior to anterior regions ($F_{3,57}=529$, $P<0.001$) (Figure 2D). In contrast, levels of LHX6 mRNA, which is also expressed by both PV and SST neurons (Georgiev et al. 2012), did not differ across the cortical regions ($F_{3,57}=1.7$, $P=0.19$) (Figure 2E).

The expression of VIP mRNA was comparable across V2, PPC, and DLPFC, but significantly lower in V1 ($F_{3,57}=43.7$, $P<0.001$) (Figure 2F). Similarly, CR mRNA, which is selectively expressed in most VIP neurons in the primate cortex (Gabbott and Bacon 1997; Lake et al. 2016), showed comparable expression levels in PPC and DLPFC, slightly but significantly lower levels in V2, and lowest levels in V1 ($F_{3,57}=72.1$, $P<0.001$) (Figure 2G).

The levels of GAD67 mRNA, which is expressed in all three GABA neuron subsets, were

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3 significantly greater in visual (V1 and V2) than association (PPC and DLPFC) regions ($F_{3,57}=10.2$,
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5 $P<0.001$) (Figure 2H).
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10 **Effect of schizophrenia on GABA neuron transcript levels in the visuospatial WM network**

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12 Relative to comparison subjects, schizophrenia subjects exhibited lower PV (-17% to -31%) and SST
13 (-30% to -51%) mRNA levels in all four cortical regions of the visuospatial WM network (Figure 3A
14 and B). Our mixed model detected a significant effect of diagnosis for PV ($F_{1,114}=8.4$, $P=0.02$) and SST
15 ($F_{1,114}=24.4$, $P<0.001$) mRNAs, but without significant diagnosis-by-region interaction effects
16 ($F_{3,114}=1.9$, $P=0.26$ and $F_{3,114}=4.0$, $P=0.07$ for PV and SST mRNAs, respectively). In contrast, VIP
17 mRNA expression levels did not show a significant effect of diagnosis ($F_{1,114}=2.2$, $P=0.41$) nor a
18 diagnosis-by-region interaction ($F_{3,114}=3.9$, $P=0.07$) (Figure 3C). These findings indicate that the effect
19 of schizophrenia on transcript levels of the GABA neuron subset-specific markers is generally similar
20 across the four cortical regions; however, given the marginal diagnosis-by-region interactions for SST
21 and VIP mRNAs, we cannot exclude the presence of modest differences in expression levels across
22 regions for these transcripts.
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38 Similar to PV and SST mRNAs, KCNS3 and GAD67 mRNA levels were significantly lower by
39 -10% to -22% ($F_{1,114}=21.6$, $P<0.001$) and by -8% to -19% ($F_{1,114}=9.3$, $P=0.02$), respectively, in
40 schizophrenia, with similar effects of illness across the four regions (diagnosis-by-region interaction
41 effect: $F_{3,114}=3.1$, $P=0.12$ and $F_{3,114}=2.3$, $P=0.25$ for KCNS3 and GAD67 mRNAs, respectively) (Figure
42 4). MOR mRNA levels were significantly higher by +25% to +66% in schizophrenia across the four
43 regions (diagnosis effect: $F_{1,114}=9.0$, $P=0.02$), without a significant regional difference in the magnitudes
44 of upregulation (diagnosis-by-region interaction effect: $F_{3,114}=1.9$, $P=0.26$) (Figure 4). For CR mRNA,
45 although the effect of diagnosis was not significant ($F_{1,114}=1.8$, $P=0.41$), we detected a significant
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3 diagnosis-by-region interaction ($F_{3,114}=5.8$, $P<0.01$), indicating a region-specific effect of schizophrenia
4 on CR expression (Figure 4). Indeed, post-hoc analysis revealed that CR levels were unaltered in V2,
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6 PPC and DLPFC, but were significantly upregulated by +41% in V1 ($t_{114}=3.4$, $P<0.001$) of subjects with
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8 schizophrenia. For LHX6 mRNA, we failed to detect a significant effect of diagnosis ($F_{1,114}=0.4$,
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10 $P=0.56$) or diagnosis-by-region interaction ($F_{3,114}=4.0$, $P=0.07$) (Figure 4).
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17 **Magnitudes of the effect of schizophrenia across cortical regions**

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19 For each transcript in each region, the magnitude of the effect of schizophrenia was calculated as the
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21 mean within-pair, schizophrenia-to-comparison subject expression ratio across the 20 pairs. Then, for
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23 each region, the eight transcripts were rank-ordered by the magnitude of the differences (Figure 4A). In
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25 all four regions, SST mRNA exhibited the largest decrease in schizophrenia, followed by PV, KCNS3
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27 and GAD67 mRNAs. MOR mRNA showed the greatest increase in schizophrenia across all four regions.
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29 The increases of CR mRNA were smaller than those of MOR mRNA and were not significant across the
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31 regions except for V1. These findings indicate that across the visuospatial WM network the effect of
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33 schizophrenia on transcript levels in PV and SST neurons is conserved across the four cortical regions,
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38 whereas the effect on CR mRNA levels is selective to V1 (Figure 4).
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Discussion

Here, we quantified expression levels of eight transcripts selectively expressed in the three separate subsets of cortical GABA neurons (Figure 4B) (Pfeffer et al. 2013; Lake et al. 2016; Tremblay et al. 2016) across four cortical regions of the visuospatial WM network from 20 matched pairs of comparison and schizophrenia subjects. Our findings are unique in that the examination of multiple regions within a well-described network revealed that 1) unaffected comparison subjects exhibit regional differences in the expression levels of these transcripts, 2) regional expression patterns are distinct across different classes of transcripts defined by their expression in each GABA neuron subset, and 3) the relative effect of schizophrenia on each transcript (except for CR) is conserved across nodes of the visuospatial WM network (Figure 4).

In this study, transcript levels were determined in total GM of specific cortical regions from the right hemisphere. Although it is not possible to completely exclude a minor contribution of glial cells to the measured transcript levels, these cells should not represent a major confound, as previous in situ hybridization studies demonstrated that all transcripts studied here are predominantly expressed in neurons, but not in glial cells, in the primate neocortex (Benson et al. 1991; Akbarian et al. 1995; Peckys and Landwehrmeyer 1999; Volk et al. 2000; Hashimoto et al. 2003; Morris et al. 2008; Georgiev et al. 2012; Joshi et al. 2015). Furthermore, the current observations in the right DLPFC are consistent with previous analyses of the left DLPFC. For example, lower PV, SST and GAD67 mRNA levels and unaltered CR mRNA levels in schizophrenia have been replicated in studies of the right or left DLPFC (Akbarian et al. 1995; Mellios et al. 2009; Fung et al. 2010). In addition, recent studies using quantitative immunohistochemistry revealed reduced GAD67 and PV protein levels and unaltered CR protein levels in the left DLPFC of schizophrenia subjects (Curley et al. 2011; Chung et al. 2016a; Enwright et al. 2016; Rocco et al. 2016).

Transcript expression patterns across WM network in the healthy state

In comparison subjects, PV and SST mRNAs exhibited decreasing and increasing posterior-to-anterior expression gradients, respectively, across the cortical regions, whereas VIP mRNA levels were comparable across the anterior three regions but significantly lower in V1 (Figure 2). The regional expression patterns of PV and KCNS3 mRNAs and of VIP and CR mRNAs were similar, consistent with their co-expression in different subsets of GABA neurons (Kubota et al. 1994; Gabbott and Bacon 1997; Georgiev et al. 2012; Lake et al. 2016). Interestingly, prior studies in non-human primates demonstrated that the densities of each GABA neuron subset differ among cortical regions (Hayes et al. 1991; Kondo et al. 1994; Conde et al. 1996; Defelipe et al. 1999). For example, the density of PV neurons was reported to be greater in early visual regions compared with DLPFC (Conde et al. 1996), consistent with our findings of decreasing PV mRNA levels from posterior to anterior regions. In addition, both the densities of SST neurons and fibers were lower in V1 than in the superior frontal cortex (Hayes et al. 1991), consistent with our observation of an increasing posterior-to-anterior gradient of SST mRNA levels. Therefore, the cross-regional patterns of transcripts appear to represent the neuronal densities of GABA neuron subsets that express those transcripts across the regions of the WM network. Consequently, the absence of regional difference in LHX6 mRNA levels across cortical regions likely reflects its expression in both PV and SST neurons (Georgiev et al. 2012). Although MOR was detected in most PV neurons and some SST neurons in the rodent hippocampus (Drake and Milner 2002; Stumm et al. 2004), the selectivity of MOR expression to any GABA neuron subsets has not been assessed in the human cortex. However, the regional pattern of MOR mRNA expression that was strikingly similar to that of SST mRNA might suggest more predominant expression of MOR in SST neurons than in other GABA neurons in human neocortex.

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3 The different regional patterns of PV, SST and VIP mRNA levels might reflect regional
4 differences in the relative number of synaptic targets for each GABA neuron subset. *Although both PV*
5 *and SST neurons promiscuously innervate most of neighboring pyramidal neurons within their axonal*
6 *arbors (Karnani et al. 2014), they furnish inhibitory synapses on distinctive subcellular domains of these*
7 *pyramidal neurons, perisomatic regions vs. distal dendrites, respectively (Figure 4B) (Tremblay et al.*
8 *2016). On the other hand, VIP neurons specifically target SST neurons and, to a lesser extent, PV*
9 *neurons within their horizontally narrow, vertically elongated axon arbors (Figure 4B) (Karnani et al.*
10 *2016; Tremblay et al. 2016).* In primates, the densities of neurons in V1 and V2 are ~3 times and ~2
11 times higher, respectively, than in DLPFC, with the density in PPC intermediate (Haug 1987; Collins et
12 al. 2010). Given that pyramidal neurons represent 75~80% of all neurons across the neocortex (Hendry
13 et al. 1987), the densities of pyramidal neuron somata would be expected to decline from
14 posterior-to-anterior regions. In our analysis, PV mRNA levels were 5.4 times, 3.4 times and 1.7 times
15 higher in V1, V2 and PPC, respectively, than in DLPFC (Figure 2A). Given that PV neurons principally
16 innervate the perisomatic region of pyramidal neurons, the regional pattern of PV mRNA levels suggests
17 that the number of PV neurons varies directly with the number of pyramidal neuron somata across
18 regions. In contrast, the posterior-to-anterior increase in SST mRNA levels (Figure 2C) suggests that the
19 ratio of SST neurons to pyramidal neurons is greater in anterior regions relative to posterior regions.
20 This apparent mismatch might reflect regional differences in the size of pyramidal neuron dendritic
21 arbors that are innervated by SST neurons. For example, in monkeys, the mean dendritic field areas of
22 pyramidal neurons were 1.3 times, 2.5~3.5 times and >5 times larger in V2, PPC and DLPFC,
23 respectively, than in V1 (Elston and Rosa 1997; Elston 2000). The total number of dendritic branches
24 per pyramidal neuron showed a similar posterior-to-anterior increase (Elston and Rosa 1997; Elston
25 2000), indicating a substantial increase in the complexity of dendritic integration by pyramidal neurons
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3 from posterior to anterior regions. Thus, more SST neurons appear to provide inhibitory inputs to a
4 relatively greater amount of pyramidal neuron dendrites in anterior than in posterior regions. Although
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from posterior to anterior regions. Thus, more SST neurons appear to provide inhibitory inputs to a relatively greater amount of pyramidal neuron dendrites in anterior than in posterior regions. Although VIP neurons innervate almost exclusively SST neurons in the mouse V1 (Pfeffer et al. 2013), several studies provided evidence for their direct inhibition of both SST and PV neurons in multiple regions, including the auditory, motor and prefrontal cortices in rodents (Donato et al. 2013; Pi et al. 2013; Lagler et al. 2016). Therefore, the relatively similar VIP mRNA levels across V2, PPC and DLPFC (Figure 2F) might reflect a similar number of targets for VIP neurons among these regions, as the increase in SST neurons from posterior to anterior regions would be balanced by the decrease in PV neurons across these regions (Hayes et al. 1991; Kondo et al. 1994; Conde et al. 1996). On the other hand, the lower VIP mRNA levels in V1 (Figure 2F) might correspond to a smaller number of VIP neuron targets compared with other regions, if VIP neurons innervate mainly SST neurons (Pfeffer et al. 2013) whose density was lowest in V1 (Hayes et al. 1991).

Transcript changes across WM network in schizophrenia

For all transcripts expressed in PV and SST neurons, the effect of schizophrenia on expression levels was markedly conserved across the four cortical regions of the WM network (Figure 4A). Although a recent study of layer 3 transcript levels in the same four cortical regions detected a lower GAD67 mRNA levels only in DLPFC of schizophrenia subjects (Hoftman et al. 2018), the change in GAD67 mRNA levels in layer 3 might not represent alterations in PV and SST neurons in total GM. Consistent with this idea, deficits in GAD67 mRNA expression in total GM have been reported in multiple cortical regions, including DLPFC, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and superior temporal gyrus (STG), of schizophrenia subjects (Akbarian et al. 1995; Woo et al. 2004; Thompson et al. 2009; Joshi et al. 2012). **The lower GAD67 mRNA levels in ACC, OFC and STG indicate that**

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3 alterations in GABA neurons are present in neocortical areas outside of the visuospatial WM network in
4 schizophrenia. Indeed, we previously found that PV, SST and GAD67 mRNA levels in total GM were
5 lower across four cortical regions (i.e., DLPFC, ACC, primary motor cortex and V1) that belong to
6 different functional domains in schizophrenia, using 9-12 matched subject pairs, which do not overlap
7 with the current 20 pairs except for one pair (Hashimoto et al. 2008b). Other groups have also reported
8 that PV and SST mRNA levels were lower in total GM of neocortical areas outside of the visuospatial
9 WM network, including ACC and OFC (Fung et al. 2014; Joshi et al. 2015; McMeekin et al. 2016).
10 Together, these findings indicate that the alteration of both PV and SST neurons is shared by a wide
11 range of cortical regions in schizophrenia.
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24 Several lines of evidence suggest that the conserved alterations of transcript levels in PV and
25 SST neurons across cortical regions are unlikely to be the consequence of other factors commonly
26 associated with schizophrenia. First, in our previous analyses, none of comorbid factors, such as the
27 presence of substance abuse/dependence at the time of death, the use of antidepressants,
28 benzodiazepine/anticonvulsant or antipsychotics at the time of death, and suicide as the cause of death,
29 had a significant effect on the expression of PV, SST, KCNS3, MOR or GAD67 mRNAs in the DLPFC
30 of schizophrenia subjects (Hashimoto et al. 2008a; Hashimoto et al. 2008b; Morris et al. 2008; Curley et
31 al. 2011; Volk et al. 2012b; Georgiev et al. 2014). Second, none of these transcripts exhibited altered
32 expression in the DLPFC of monkeys chronically exposed to antipsychotics at clinically relevant doses
33 (Volk et al. 2000; Hashimoto et al. 2003; Hashimoto et al. 2008a; Morris et al. 2008; Volk et al. 2012b;
34 Georgiev et al. 2014). Third, the comparable magnitudes of expression changes of each transcript across
35 the four cortical regions argue against a potential influence of antipsychotics on these transcripts, as any
36 effects of antipsychotics would be likely to differ across these regions, which markedly differ in the
37 densities of both dopamine axons (Lewis et al. 1987; Gaspar et al. 1989) and dopamine receptors (Lidow
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3 et al. 1989). Finally, using the current data, we confirmed that none of the comorbid factors had a
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5 significant effect on the transcript levels in any cortical region of schizophrenia subjects (Supplementary
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7 Table 3).
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10 11 12 **Potential pathogenic mechanisms underlying transcript changes** 13

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15 Instead, our findings are more likely to reflect pathogenic mechanisms affecting gene expression that are
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17 common to PV and SST neurons and conserved across cortical regions. For example, the expression of
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19 PV, SST, KCNS3 and GAD67 mRNAs are all activity dependent (Marty and Onteniente 1997; Lee et al.
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21 2015; Cohen et al. 2016; Mardinly et al. 2016). Thus, their downregulation in schizophrenia likely
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23 reflects the reduced activity of PV and SST neurons, which might be secondary to lower activity in
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25 neighboring pyramidal neurons which provide the largest source of excitatory drive to PV and SST
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27 neurons (Tremblay et al. 2016). Consistent with this idea, the density of pyramidal neuron dendritic
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29 spines, the site of most excitatory synapses, was found to be decreased across various cortical regions,
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31 including the DLPFC, orbitofrontal cortex, temporal association cortex and hippocampus, in subjects
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33 with schizophrenia (Glausier and Lewis 2013). Although spine density was not significantly lower in V1
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35 of schizophrenia subjects (Glantz and Lewis 2000), excitatory feedback from other cortical regions
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37 might be reduced in this region. Another, but not mutually exclusive, possibility is that alterations in PV
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39 and SST neurons are due to shared disruptions in their early development caused by genetic and/or
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41 environmental insults, as these neurons, but not VIP neurons, have a common site of origin and common
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43 regulators of their proliferation, migration and phenotypic specification (Volk and Lewis 2014).
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45 Interestingly, transcripts of some of these regulators, including transcription factors and chemokine
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47 receptors, were found to be upregulated in the DLPFC of subjects with schizophrenia (Volk et al. 2015;
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49 Volk et al. 2016). These changes might reflect compensatory responses to maintain normal development
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3 of these neurons in the face of insults.
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8 **Functional implications**

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10 The differences in transcript levels observed across cortical nodes of the visuospatial WM network in
11 schizophrenia (Figure 4A and B) might contribute to the neuronal substrate for WM impairments in the
12 illness. Both PV and SST neurons play important roles in generating neural oscillations in wide
13 frequency ranges (Buzsaki and Wang 2012; Amilhon et al. 2015; Chen et al. 2017; Veit et al. 2017) and
14 synchronization of these oscillations across multiple cortical regions regulates information processing in
15 distributed neuronal networks (Bastos et al. 2015; Spellman et al. 2015), including the visuospatial WM
16 network (Palva et al. 2010). In addition, a suppression of SST neuron activities in V1 was shown to
17 reduce context-dependent visually induced oscillations (Chen et al. 2017; Veit et al. 2017). Therefore,
18 alterations of PV and SST neurons across the cortical regions and of VIP neurons in V1, which primarily
19 inhibit local SST neurons (Pfeffer et al. 2013), could have a profound impact on the generation and
20 synchronization of cortical oscillations across the visuospatial WM network, contributing to the WM
21 impairments in schizophrenia.
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37 As both PV- and SST-positive GABA neuron subsets can be further divided into subtypes with
38 distinctive morphological and functional properties and laminar distributions, the identification of
39 affected subtypes will be important for further understanding the pathophysiology of cortical network
40 dysfunction in schizophrenia. For example, a minority of SST neurons directly connects distant cortical
41 regions via long-range projections and could contribute to the mechanism for synchronizing oscillations
42 among multiple cortical regions (Tomioka and Rockland 2007; Tamamaki and Tomioka 2010).
43 Consequently, the lower SST mRNA levels across the cortical regions might reflect alterations of direct
44 inter-regional inhibition within the WM network in schizophrenia. However, this possibility remains
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3 speculative due to the absence of a specific marker of long-range projecting SST neurons in the human
4 cortex. Interestingly, a recent single cell RNA sequencing study of mouse cortical neurons reported
5 multiple transcripts that were differentially expressed among different subtypes of each GABA neuron
6 subset (Paul et al. 2017). These transcripts could be used in the future studies to identify affected
7 subtypes of PV and SST neurons across cortical regions in schizophrenia.
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17 **Conclusions**

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19 In summary, our analyses of transcript levels across cortical regions in the visuospatial WM network
20 revealed that the transcripts associated with each GABA neuron subset had distinct regional expression
21 patterns in comparison subjects, suggesting that the contribution of each GABA neuron subset to the
22 inhibitory regulation of local circuitry normally differs across cortical regions of the WM network. We
23 also found that each transcript expressed in PV and SST neurons was affected in a similar fashion across
24 these regions in subjects with schizophrenia (Figure 4), suggesting that alterations of PV and SST
25 neurons represent a shared feature across diverse cortical regions, including those for the visuospatial
26 WM network.
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Acknowledgements

This work was supported by Japan Society for the Promotion of Science (Grants-in-Aid 15H01280, 16H05372 to TH) and National Institutes of Health (P50 MH103204 to DAL).

David A. Lewis currently receives investigator-initiated research support from Pfizer. In 2016-2018, he served as a consultant in the areas of target identification and validation and new compound development to Merck. The other authors declare no conflict of interest.

For Peer Review

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18 cingulate cortex in schizophrenia and bipolar disorder. *Arch Gen Psychiatry* 61:649-657.
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Table 1: Summary of demographic and postmortem characteristics of human subjects

Parameter	Unaffected Comparison Subjects	Subjects with Schizophrenia	Statistics
Number	20	20	N/A
Sex, Male/Female	14/6	14/6	N/A
Race, White/Black	15/5	14/6	$\chi^2_1 = 0.1$; P = 0.72
Age, Years	45.4 ± 11.6	44.3 ± 10.4	$t_{38} = -0.3$; P = 0.74
PMI, Hours	15.4 ± 5.8	14.3 ± 6.4	$t_{38} = -0.6$; P = 0.57
RIN	8.3 ± 0.5	8.3 ± 0.6	$t_{38} = -0.2$; P = 0.87
Brain pH	6.7 ± 0.3	6.5 ± 0.3	$t_{38} = -1.7$; P = 0.10
Storage Time at -80°C, Months	111.9 ± 41.3	113.0 ± 48.8	$t_{38} = 0.1$; P = 0.94

Values are n or mean ± SD.

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

Captions to Figures

Figure 1. Cortical regions for RNA extraction.

Schematic medial (left) and lateral (right) views of the right hemisphere of the human brain (top). Vertical bars indicate the approximate position of coronal blocks (bottom). The locations of sampled regions are represented by different patterns. V1, primary visual cortex; V2, association visual cortex; PPC, posterior parietal cortex; DLPFC, dorsolateral prefrontal cortex; cs, calcarine sulcus; ips, intraparietal sulcus; sfs, superior frontal sulcus.

Figure 2. Expression levels of transcripts selective to GABA neuron subsets across the four cortical regions of the visuospatial WM network in unaffected comparison subjects.

In each graph, transcript name is at the top center and the mixed model result at the top right. Across regions, transcript levels of individual subjects are plotted as open circles and group means as horizontal lines. For each transcript, regions not sharing the same [horizontal bar](#) are statistically different on post-hoc comparisons. V1, primary visual cortex; V2, association visual cortex; PPC, posterior parietal cortex; DLPFC, dorsolateral prefrontal cortex.

Figure 3. Transcript levels in three GABA neuron subsets across the four cortical regions of the visuospatial WM network in subjects with schizophrenia and matched comparison subjects.

Individual comparison and schizophrenia subjects are represented by open and filled circles, respectively, and the members of each pair are connected by lines. Horizontal bars indicate group means. [The inset in panel A shows PV mRNA levels in the DLPFC with the expanded Y-axis for a better illustration of the effect of schizophrenia.](#) V1, primary visual cortex; V2, association visual cortex; PPC, posterior parietal cortex; DLPFC, dorsolateral prefrontal cortex.

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5 **Figure 4. Summary of findings.**
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8 A: Eight GABA neuron transcripts were rank-ordered by the magnitude of change in schizophrenia of
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10 their expression levels in each of the four cortical regions of the visuospatial WM network. Note that the
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12 rank orders are remarkably similar across the four regions; the only exception is the reversal of GAD67
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16 parietal cortex; DLPFC, dorsolateral prefrontal cortex.
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19 B: PV-, SST- and VIP-positive subsets of GABA neurons are schematically presented in green, red and
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21 dark-blue, respectively (left). Broken lines connect each GABA neuron subset and selectively expressed
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23 transcripts (middle). For each transcript, the effect of schizophrenia on its expression level across the
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25 WM network is indicated by arrows (right). †significant diagnosis effect, §significant
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27 diagnosis-by-region interaction effect.
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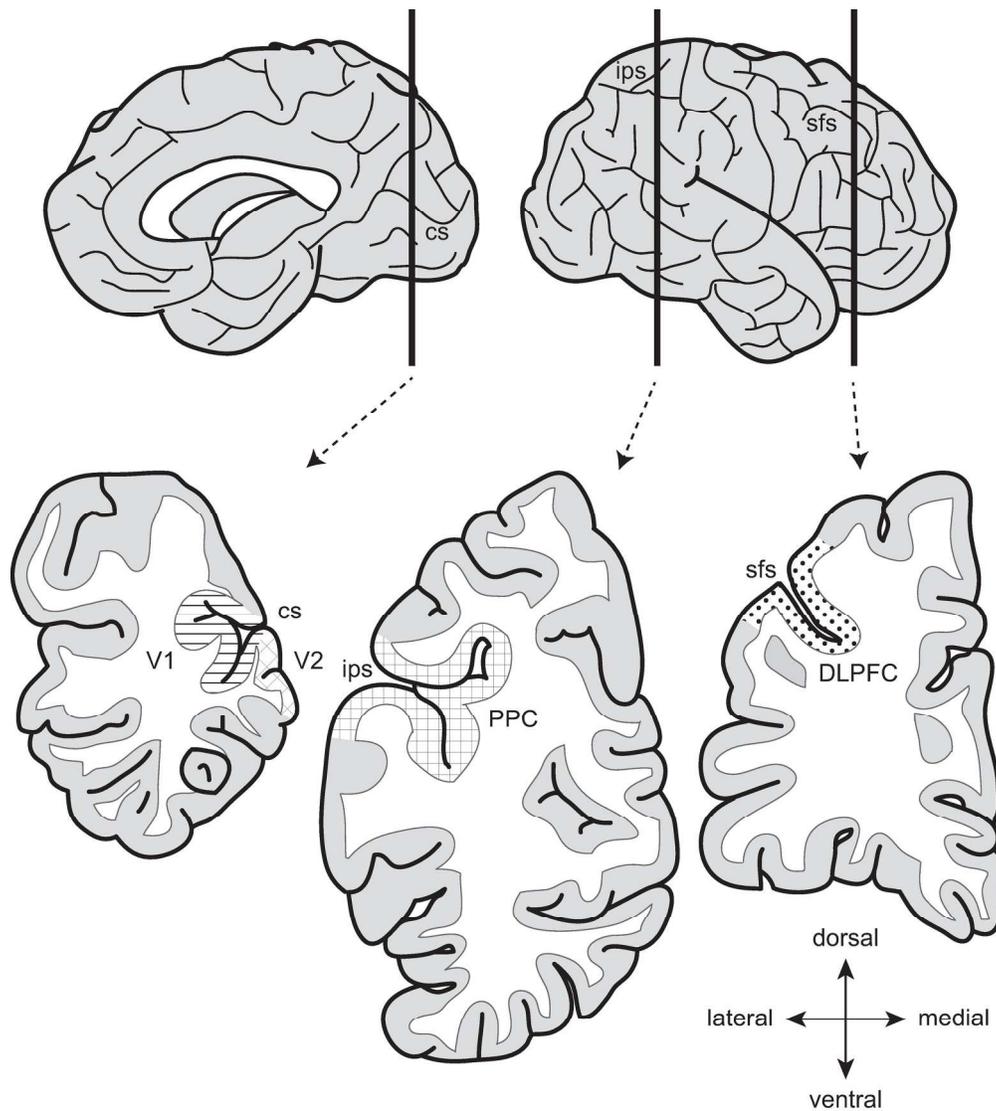


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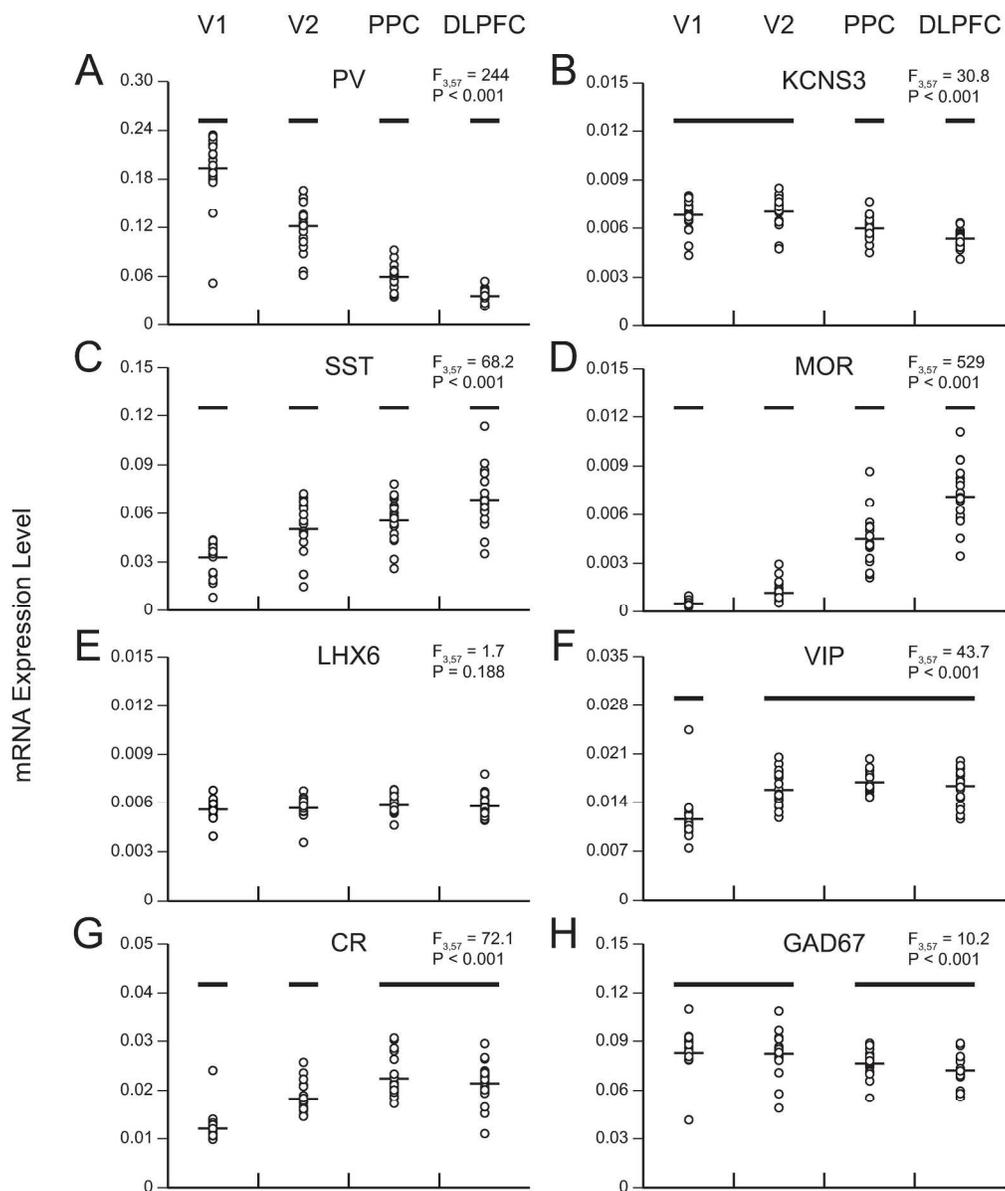


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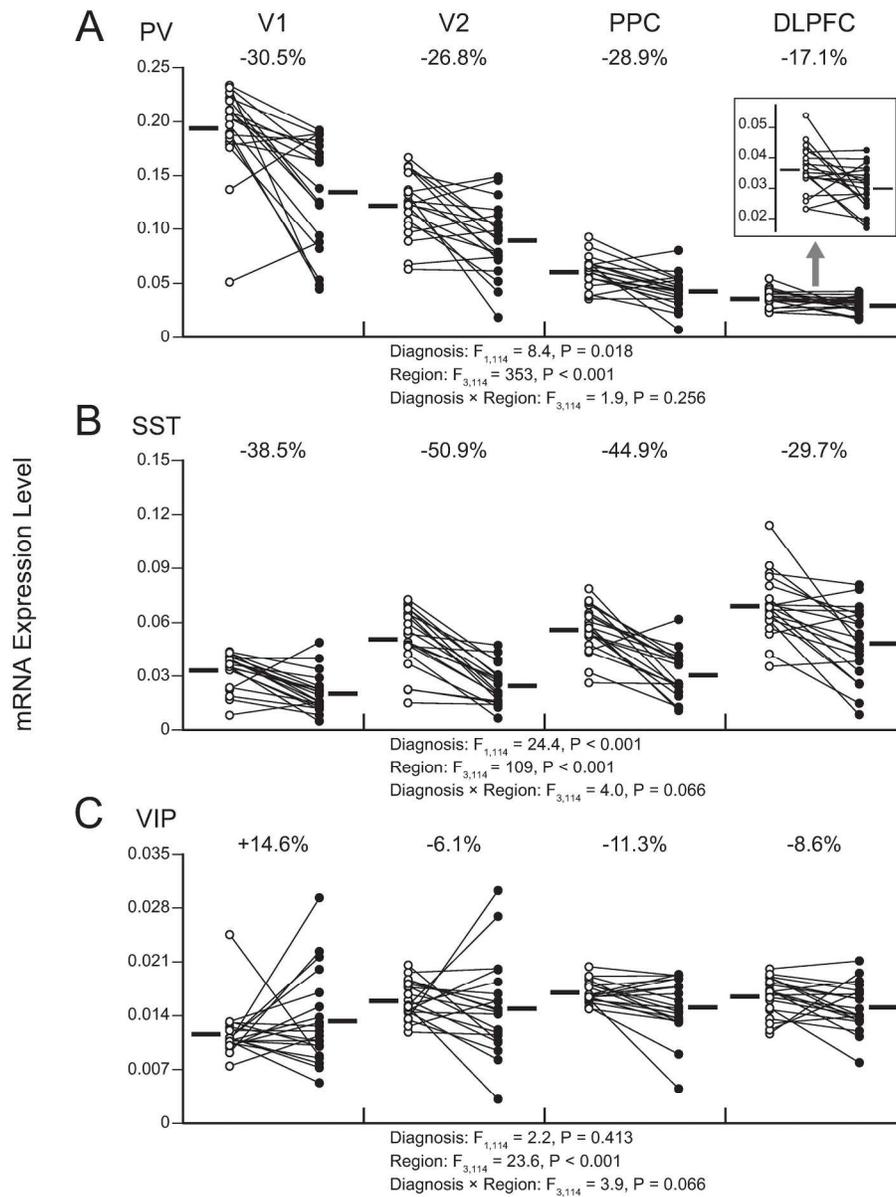


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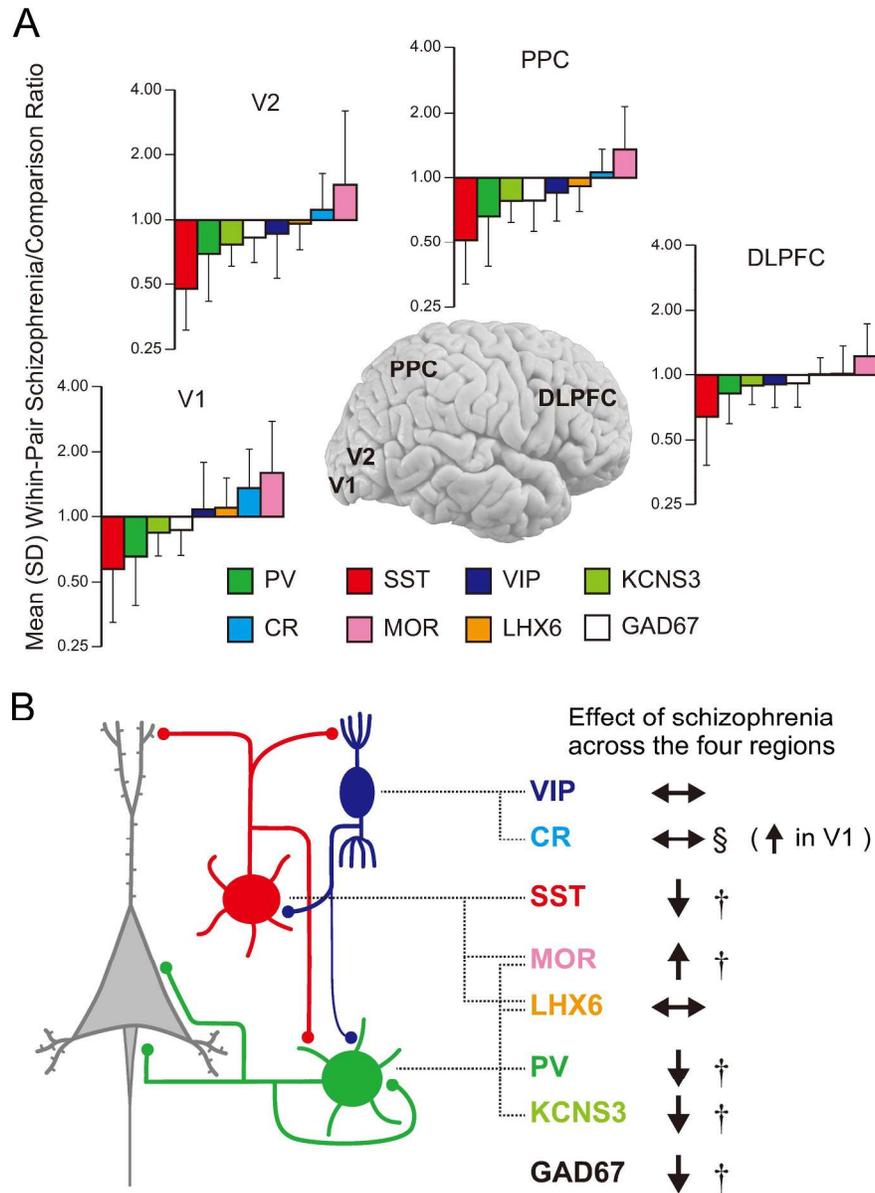


Figure 4. Summary of findings.

A: Eight GABA neuron transcripts were rank-ordered by the magnitude of change in schizophrenia of their expression levels in each of the four cortical regions of the visuospatial WM network. Note that the rank orders are remarkably similar across the four regions; the only exception is the reversal of GAD67 and VIP mRNAs in DLPFC. V1, primary visual cortex; V2, association visual cortex; PPC, posterior parietal cortex; DLPFC, dorsolateral prefrontal cortex.

B: PV-, SST- and VIP-positive subsets of GABA neurons are schematically presented in green, red and dark blue, respectively (left). Broken lines connect each GABA neuron subset and selectively expressed transcripts (middle). For each transcript, the effect of schizophrenia on its expression level across the WM network is indicated by arrows (right). †significant diagnosis effect, §significant diagnosis-by-region interaction effect.

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For Peer Review

Supplementary Table 1. Demographic, postmortem, and clinical characteristics of human subjects used in this study.

Unaffected comparison subjects									Subjects with schizophrenia								
Pair	Case	Sex / Race	Age	PMI ^a	Storage Time ^b	RIN ^c	Brain pH	Cause of Death ^d	Case	DSM IV diagnosis	Sex / Race	Age	PMI ^a	Storage Time ^b	RIN ^c	Brain pH	Cause of Death ^d
1	1196	F/W	36	14.5	108	8.2	6.4	Asphyxiation	537	Schizoaffective disorder	F/W	37	14.5	212	8.6	6.7	Suicide by hanging
2	546	F/W	37	23.5	211	8.6	6.7	ASCVD	587	Chronic undifferentiated schizophrenia ^{e,f,h,k}	F/B	38	17.8	204	9.0	7.0	Myocardial hypertrophy
3	681	M/W	51	11.6	191	8.9	7.2	Hypertrophic cardiomyopathy	640	Chronic paranoid schizophrenia ^{e,f,g}	M/W	49	5.2	196	8.4	6.9	Pulmonary embolism
4	727	M/B	19	7.0	184	9.2	7.2	Trauma	829	Schizoaffective disorder ^{e,h,i,n}	M/W	25	5.0	165	9.3	6.8	Suicide by salicylate overdose
5	1047	M/W	43	13.8	126	9.0	6.6	ASCVD	1209	Schizoaffective disorder ^f	M/W	35	9.1	106	8.7	6.5	Suicide by diphenhydramine overdose
6	1092	F/B	40	16.6	119	8.0	6.8	Mitral valve prolapse	1178	Schizoaffective disorder ^{e,f,h}	F/B	37	18.9	110	8.4	6.1	Pulmonary embolism
7	1122	M/W	55	15.4	115	7.9	6.7	Cardiac tamponade	1105	Schizoaffective disorder ^{e,f}	M/W	53	7.9	118	8.9	6.2	ASCVD
8	1284	M/W	55	6.4	95	8.7	6.8	ASCVD	1188	Undifferentiated schizophrenia ^{e,f,k,n}	M/W	58	7.7	109	8.4	6.2	ASCVD
9	1191	M/B	59	19.4	108	8.4	6.2	ASCVD	1263	Undifferentiated schizophrenia ^{e,f,g,j}	M/W	62	22.7	98	8.5	7.1	Accidental asphyxiation
10	10003	M/W	49	21.2	108	8.4	6.5	Trauma	1088	Undifferentiated schizophrenia ^{e,f,g,i,m}	M/W	49	21.5	119	8.1	6.5	Accidental combined drug overdose
11	1247	F/W	58	22.7	100	8.4	6.4	ASCVD	1240	Undifferentiated schizophrenia ^{e,f,j}	F/B	50	22.9	101	7.7	6.3	ASCVD
12	1099	F/W	24	9.1	118	8.6	6.5	Cardiomyopathy	10023	Disorganized schizophrenia ^{f,h}	F/B	25	20.1	99	7.4	6.7	Suicide by drowning
13	1307	M/B	32	4.8	90	7.6	6.7	ASCVD	10024	Paranoid schizophrenia	M/B	37	6.0	99	7.5	6.1	ASCVD
14	1391	F/W	51	7.8	75	7.1	6.6	ASCVD	1189	Schizoaffective disorder ^{e,f,g,h,k}	F/W	47	14.4	109	8.3	6.4	Suicide by combined drug overdose
15	1159	M/W	51	16.7	112	7.6	6.5	ASCVD	1296	Undifferentiated schizophrenia ^{e,f,g}	M/W	48	7.8	92	7.3	6.5	Pneumonia
16	1326	M/W	58	16.4	86	8.0	6.7	ASCVD	1314	Undifferentiated schizophrenia ^{f,g}	M/W	50	11.0	89	7.2	6.2	ASCVD
17	1268	M/B	49	19.9	97	7.9	7.1	ASCVD	1230	Undifferentiated schizophrenia ^{e,f,g}	M/W	50	16.9	102	8.2	6.6	Suicide by doxepin overdose
18	1386	M/W	46	21.2	76	8.3	6.7	ASCVD	1420	Schizoaffective disorder ^{e,f,g,k,l,n}	M/W	47	23.4	70	8.2	6.7	Suicide by jump
19	1372	M/W	37	20.5	80	9.0	6.6	Asphyxiation	1581	Paranoid schizophrenia ^{e,f,g,l,m}	M/W	32	18.4	40	9.0	6.8	ASCVD
20	1583	M/W	58	19.1	39	8.2	6.7	Trauma	1686	Paranoid schizophrenia ^{e,f,g,h,k}	M/B	56	14.1	23	8.3	6.2	ASCVD

a, PMI, postmortem interval (hours); b, Storage time (months) at -80°C; c, RIN, RNA integrity number; d, ASCVD, atherosclerotic coronary vascular disease; e, nicotine use at time of death; f, antipsychotic medication use at time of death; g, antidepressant use at time of death; h, use of benzodiazepines or sodium valproate at time of death; i, alcohol dependence, current at time of death; j, alcohol dependence, in remission at time of death; k, alcohol abuse, in remission at time of death; l, other substance dependence, current at time of death; m, other substance abuse, current at time of death; n, other substance abuse, in remission at time of death

Supplementary Table 2 Real-time PCR primer sets.

Gene	Species	Accession #	Position	Forward Primer (F) Reverse Primer (R)
Parvalbumin	Human	NM_002854	112-210	(F) CTACCGACTCCTTCGACCA (R) CCTTGTCCAGCATGTGAAA
KCNS3	Human	NM_002252	1260-1385	(F) AAGGTGGTCCAGATCCTACG (R) AAGCAGAAGCCCAACTTCAT
Somatostatin	Human	NM_001048	319-411	(F) ATGCCCTGGAACCTGAAGAT (R) CCATAGCCGGGTTTGAGTTA
μ opioid receptor	Human	NM_000914	1359-1416	(F) GCCTTGGTTACAATCCCAGA (R) CAATGCAGAAGTGCCAAGAA
Lhx6	Human	NM_014368	714-809	(F) CGACACCATGATTGAGAACC (R) TTGGGTTGACTGTCCTGTTC
Vasoactive intestinal peptide	Human	NM_003381	252-396	(F) CCTCTTTACAGGGCACCTTC (R) CATCATAATAGGGTGTGTCAATTTTC
Calretinin	Human	NM_001740	405-549	(F) AGCGCCGAGTTTATGGAG (R) GGGTGTATTCCTGGAGCTTG
GAD67	Human	NM_000817	2495-2580	(F) GTTTCCCGCTCCAAGAGAAT (R) TGGAGTTGTTGGACAAGCTG
Beta Actin	Human	NM_001101	1146-1246	(F) GATGTGGATCAGCAAGCA (R) AGAAAGGGTGTAACGCAACTA
Cyclophilin	Human	NM_021130	165-290	(F) GCAGACAAGTCCCAAAG (R) GAAGTCACCACCCTGACAC

Supplementary Table 3. Comorbid factor analysis for individual transcripts in all regions.

Region	Transcript	Substance abuse				Benzodiazepine					
		/dependence		Antidepressant		/Anticonvulsant		Antipsychotic		Suicide	
		t	P*	t	P*	t	P*	t	P*	t	P*
DLPFC	GAD67	0.5	1.00	0.6	1.00	-0.9	1.00	1.6	0.70	-0.1	1.00
	PV	-0.6	1.00	-0.9	1.00	1.1	1.00	-0.5	1.00	1.4	1.00
	KCNS3	-0.9	1.00	-0.3	1.00	0.5	1.00	1.2	1.00	-0.7	1.00
	SST	-0.3	1.00	1.8	0.65	-1.0	1.00	1.9	0.55	-0.4	1.00
	MOR	1.7	0.88	-0.3	1.00	0.5	1.00	-0.7	1.00	0.8	1.00
	LHX6	0.7	1.00	0.9	1.00	-0.8	1.00	0.8	1.00	1.1	1.00
	CR	0.2	1.00	0.3	1.00	1.6	0.96	0.2	1.00	0.0	1.00
	VIP	0.0	1.00	2.0	0.52	-0.8	1.00	1.7	0.69	-0.3	1.00
PPC	GAD67	-0.4	1.00	0.4	1.00	0.2	1.00	0.4	1.00	0.9	1.00
	PV	-0.8	1.00	-0.5	1.00	0.6	1.00	0.0	1.00	1.7	0.78
	KCNS3	-1.6	0.97	-0.4	1.00	1.3	1.00	0.6	1.00	0.7	1.00
	SST	-0.9	1.00	1.2	1.00	0.2	1.00	1.4	1.00	-0.4	1.00
	MOR	1.3	1.00	1.2	1.00	-0.3	1.00	0.0	1.00	1.3	1.00
	LHX6	0.0	1.00	1.1	1.00	-0.3	1.00	0.6	1.00	1.7	0.78
	CR	-0.8	1.00	-0.1	1.00	0.7	1.00	0.0	1.00	-0.6	1.00
	VIP	-1.0	1.00	1.0	1.00	-0.5	1.00	1.1	1.00	0.1	1.00
V2	GAD67	0.7	1.00	1.1	1.00	0.0	1.00	0.4	1.00	0.4	1.00
	PV	-0.9	1.00	0.0	1.00	0.2	1.00	0.3	1.00	0.7	1.00
	KCNS3	-0.4	1.00	0.7	1.00	0.2	1.00	0.0	1.00	-0.4	1.00
	SST	-0.5	1.00	1.3	1.00	0.2	1.00	0.7	1.00	-0.5	1.00
	MOR	2.1	0.42	1.0	1.00	-0.6	1.00	-0.8	1.00	1.8	0.69
	LHX6	0.9	1.00	1.5	1.00	-0.3	1.00	-0.1	1.00	1.1	1.00
	CR	0.9	1.00	1.4	1.00	-0.5	1.00	0.0	1.00	0.7	1.00
	VIP	-0.2	1.00	2.0	0.44	-1.1	1.00	1.3	1.00	-0.1	1.00
V1	GAD67	2.0	0.34	0.8	1.00	1.3	1.00	-0.5	1.00	0.5	1.00
	PV	0.2	1.00	-0.5	1.00	0.7	1.00	-0.8	1.00	1.2	1.00
	KCNS3	1.7	0.54	0.9	1.00	1.0	1.00	-0.6	1.00	-0.1	1.00
	SST	-0.1	1.00	1.2	1.00	-0.7	1.00	0.5	1.00	-0.3	1.00
	MOR	2.4	0.24	1.0	1.00	-0.9	1.00	0.4	1.00	1.5	1.00
	LHX6	2.2	0.28	1.7	0.75	-0.4	1.00	-0.2	1.00	1.4	1.00
	CR	0.9	1.00	1.7	0.75	-0.5	1.00	0.7	1.00	-0.1	1.00
	VIP	0.1	1.00	2.2	0.35	-1.5	1.00	1.5	1.00	-0.4	1.00

*P values corrected for multiple comparisons using the Holm simultaneous inference procedure