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

Supplemental Information

Supplemental Methods

Our experimental design controlled for batch effects within subject pairs and across regions within subjects (i.e., all transcripts from all regions of both subjects in a pair were assayed on the same qPCR plate), but necessarily limited the number of transcripts that could be studied, excluding the possibility of examining other glutamate or GABA receptor transcripts. Thus, the functionally-analogous markers of glutamate and GABA neurotransmission were selected for the following reasons: glutaminase (GLS1) and the 67 kD isoform of glutamic acid decarboxylase (GAD67) are the enzymes that synthesize most cortical glutamate and GABA, respectively (1-3); the vesicular glutamate (vGLUT1) and GABA (vGAT) transporters package their respective neurotransmitters into presynaptic vesicles in cortical axon terminals (4,5); EAAT2, the glutamate transporter localized to astrocytes, is responsible for the removal of most of the glutamate from the synaptic space (6); GAT1, the GABA transporter that is abundant in the neocortex and localized to neuronal and glial membranes (7,8), is responsible for the removal of most GABA from the synapse (9); GRIA2 is enriched in layer 3 of primate neocortex (10), is present in the majority of neocortical AMPA receptors, and is important for AMPA receptor calcium permeability (11); GRIN1 is required to assemble a functional NMDA receptor (12); and GABRG2 is present in the majority of $GABA_A$ receptors and is required to render a synaptically located ionotropic GABA receptor functional (13).

In addition to prior evidence, the use of the internal reference genes (Beta-Actin [ACTB] and Cyclophilin-A [CYCLO]) was supported by our findings of the absence of region by diagnosis interaction ($F_{3,114}$=1.1; p=0.35) effect in transcript expression levels for beta actin normalized against cyclophilin using the mixed model described in the main text. Furthermore, expression ratios of these normalizers differed between subject groups by only 1.6-7.1% across the four regions, and there was no significant effect of diagnosis on normalizer transcript expression levels when corrected for multiple comparisons ($F_{1,113}$=4.0; p>0.10). Finally, the opposite differences

between diagnostic groups in the regional patterns of glutamate and GABA transcript expression support the absence of any systematic regional or diagnostic effects of normalizers.

For the composite measures, the glutamate composite score was computed as the sum of the normalized (Z-score) expression levels for each of the five transcripts. For each expression ratio value, we calculated the Z-score = $((X - \mu)/ \sigma)$, where μ is the mean of the group, σ is the standard deviation of the group, and X is the individual transcript measure. Then, we summed the Z-scores of all glutamate transcripts across subjects within a group for each region, an approach that provides equal weight to each transcript. The same approach was used for calculating the GABA composite score for the four GABA transcripts measured.

As noted in the Methods and Materials, the potential confounding effect of antipsychotic medications, nicotine or other substances of abuse, suicide and other factors frequently comorbid with schizophrenia were also examined **(Supplemental Table S3)**. Regression or two-sample t-tests were used to check the relationship of the expression level in schizophrenia to each of the comorbid factors, where the expression level in schizophrenia subjects was contrasted with the matched unaffected comparison subject (i.e., (S-C)/C).

Supplemental Table S1. 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	pH	Cause of Death	Case	DSM IV diagnosis	Sex/ Race	Age	PMI[a]	Storage Time[b]	RIN	pH	Cause of Death	Tobacco ATOD	Anti-psychotic ATOD	Anti-depressant ATOD	Benzodiazepine/ Anticonvulsant ATOD
1	1196	F/W	36	14.5	132	8.2	6.4	Asphyxiation	537	Schizoaffective disorder	F/W	37	14.5	236	8.6	6.7	Suicide by hanging	Unknown	No	No	No
2	546	F/W	37	23.5	235	8.6	6.7	ASCVD	587	Chronic undifferentiated schizophrenia; AAR	F/B	38	17.8	228	9.0	7.0	Myocardial hypertrophy	Yes	Yes	No	Yes
3	681	M/W	51	11.6	215	8.9	7.2	Hypertrophic cardio-myopathy	640	Chronic paranoid schizophrenia	M/W	49	5.2	220	8.4	6.9	Pulmonary embolism	Unknown	Yes	Yes	No
4	852	M/W	54	8.0	183	9.1	6.8	Cardiac tamponade	781	Schizoaffective disorder; ADR	M/B	52	8.0	198	7.7	6.7	Peritonitis	Yes	Yes	Yes	No
5	1047	M/W	43	13.8	150	9.0	6.6	ASCVD	1209	Schizoaffective disorder	M/W	35	9.1	130	8.7	6.5	Suicide by diphenhydramine overdose	No	Yes	No	No
6	1092	F/B	40	16.6	143	8.0	6.8	Mitral valve prolapse	1178	Schizoaffective disorder	F/B	37	18.9	135	8.4	6.1	Pulmonary embolism	Yes	Yes	No	Yes
7	1122	M/W	55	15.4	140	7.9	6.7	Cardiac tamponade	1105	Schizoaffective disorder	M/W	53	7.9	142	8.9	6.2	ASCVD	Yes	Yes	No	No
8	1284	M/W	55	6.4	119	8.7	6.8	ASCVD	1188	Undifferentiated schizophrenia; AAR; OAR	M/W	58	7.7	133	8.4	6.2	ASCVD	Yes	Yes	No	No
9	1191	M/B	59	19.4	132	8.4	6.2	ASCVD	1263	Undifferentiated schizophrenia; ADR	M/W	62	22.7	122	8.5	7.1	Accidental asphyxiation	Yes	Yes	Yes	No
10	10003	M/W	49	21.2	132	8.4	6.5	Trauma	1088	Undifferentiated schizophrenia; ADC; OAC	M/W	49	21.5	144	8.1	6.5	Accidental combined drug overdose	Yes	Yes	Yes	No
11	1247	F/W	58	22.7	124	8.4	6.4	ASCVD	1240	Undifferentiated schizophrenia; ADR	F/B	50	22.9	125	7.7	6.3	ASCVD	Yes	Yes	No	No
12	1099	F/W	24	9.1	142	8.6	6.5	Cardiomyopathy	10023	Disorganized schizophrenia	F/B	25	20.1	123	7.4	6.7	Suicide by drowning	No	Yes	No	Yes
13	1307	M/B	32	4.8	114	7.6	6.7	ASCVD	10024	Paranoid schizophrenia	M/B	37	6.0	123	7.5	6.1	ASCVD	No	No	No	No
14	1391	F/W	51	7.8	100	7.1	6.6	ASCVD	1189	Schizoaffective disorder; AAR	F/W	47	14.4	133	8.3	6.4	Suicide by combined drug overdose	Yes	Yes	Yes	Yes
15	1159	M/W	51	16.7	136	7.6	6.5	ASCVD	1296	Undifferentiated schizophrenia	M/W	48	7.8	116	7.3	6.5	Pneumonia	Yes	Yes	Yes	No
16	1326	M/W	58	16.4	110	8.0	6.7	ASCVD	1314	Undifferentiated schizophrenia	M/W	50	11.0	113	7.2	6.2	ASCVD	No	Yes	Yes	No
17	1268	M/B	49	19.9	121	7.9	7.1	ASCVD	1230	Undifferentiated schizophrenia	M/W	50	16.9	127	8.2	6.6	Suicide by doxepin overdose	Yes	Yes	Yes	No
18	1386	M/W	46	21.2	100	8.3	6.7	ASCVD	1420	Schizoaffective disorder; AAR; ODC; OAR	M/W	47	23.4	94	8.2	6.8	Suicide by jump	Yes	Yes	Yes	No
19	1372	M/W	37	20.5	104	9.0	6.6	Asphyxiation	1581	Paranoid schizophrenia; ODC; OAC	M/W	32	18.4	64	9.0	6.8	ASCVD	Yes	Yes	Yes	No
20	1583	M/W	58	19.1	63	8.2	6.8	Trauma	1686	Schizophrenia, paranoid type; AAR	M/B	56	14.1	47	8.3	6.2	ASCVD	Yes	Yes	Yes	Yes

[a] PMI, postmortem interval (hours); [b] Storage time (months) at -80C; [c] First degree relative with schizophrenia; Other abbreviations: ASCVD, arteriosclerotic cardiovascular disease; MCA, middle cerebral artery; ATOD, at time of death; ADC, alcohol dependence, current at time of death; ADR, alcohol dependence, in remission at time of death; AAC, alcohol abuse, current at time of death; AAR, alcohol abuse, in remission at time of death; ODC, other substance dependence, current at time of death; ODR, other substance dependence, in remission at time of death; OAC, other substance abuse, current at time of death; OAR, other substance abuse, in remission at time of death; U, unknown; ISP, index of social position; M, male; F, female; W, white; B, black.

Supplemental Table S2. qPCR Primer Design.

Gene	Species	Accession #	Position	Forward Primer (F) Reverse Primer (R)
EAAT2 (SLC1A2) Isoform 1	Human	NM_004171	433-502	(F) GAGGCGCTAAAAGGGCTTAC (R) GGGAGCGGTATTTAAGAGGAG
GABRG2	Human	NM_000816	1527-1644	(F) AAATGAATAATGCTACACACCTTCA (R) CAAGCTCCTGTTCGACAATC
GAD67 (GAD1)	Human	NM_000817	2495-2550	(F) GTTTCCCGCTCCAAGAGAAT (R) TGGAGTTGTTGGACAAGCTG
GAT1 (SLC6A1)	Human	NM_003042	936-1065	(F) CTGGACTGGAAAGGTGGTCTA (R) GCGGAAGTTGGGTGTGAT
GLS1	Human	NM_014905	1945-2020	(F) GCATATACTGGAGATGTGGTCGC (R) AATCATAGTCCCGCTGTTCC
GRIA2; FLIP (Variants 1 and 3)	Human	NM_000826	2719-2850	(F) GGCATCGCAACACCTAAA (R) CTTGGCTCCACATTCACCT
GRIN1	Human	NM_000832	2317-2402	(F) TCCCTGTCCATCCTCAAGTC (R) CGCGAGTCACATTCCTGATA
vGAT	Human	NM_080552	1262-1347	(F) CAGCGAGTTCCACTGCAT (R) AGGTGAGGTAGGCGACGA
vGLUT1	Human	NM_020309	436-559	(F) GGTTTTCGGCTTTGCTATTG (R) ATGTGACGCCCTCTACCAAC
ACTB	Human	NM_001101	1146-1246	(F) GATGTGGATCAGCAAGCA (R) AGAAAGGGTGTAACGCAACTA
CYCLO	Human	NM_021130	159-284	(F) GCAGACAAGGTCCCAAAG (R) GAAGTCACCACCCTGACAAC

Supplemental Table S3. Comorbid factor analysis for individual transcripts in all regions

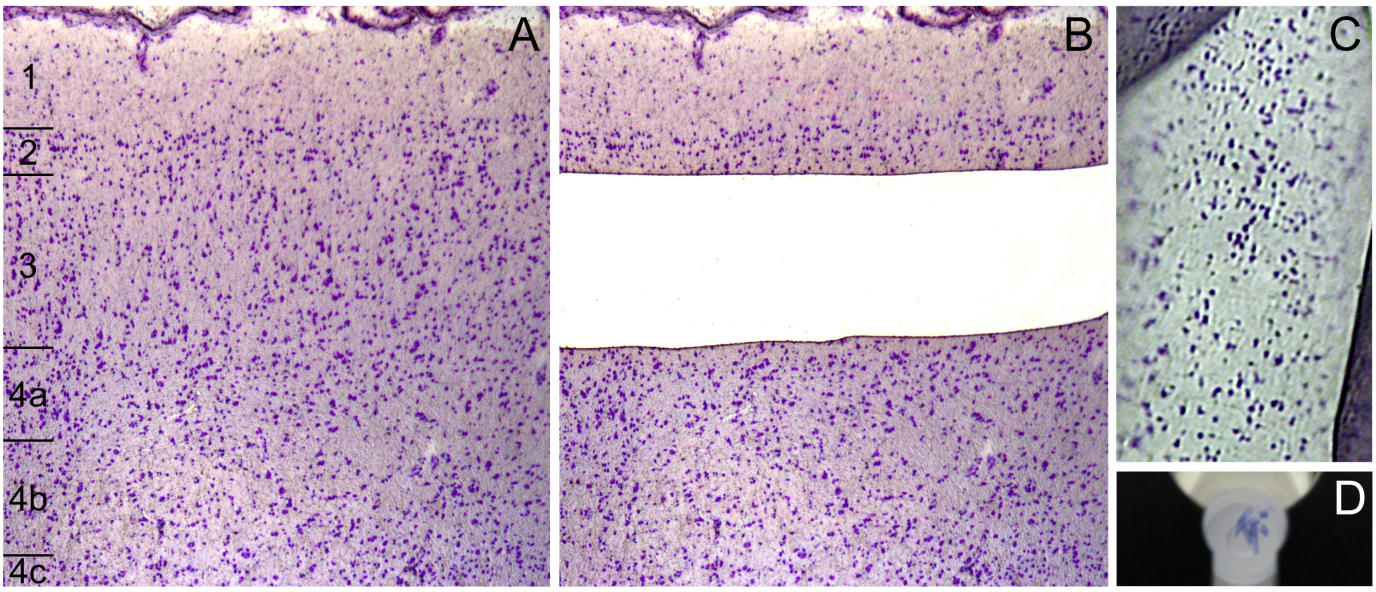
Region	Transcript	Suicide		Antipsychotic		Antidepressant		Anticonvulsant Benzodiazepine		Nicotine	
		t	P*	t	P*	t	P*	t	P*	t	P*
DLPFC	EAAT2	0.9	0.91	0.5	0.87	-0.8	0.64	0.4	0.82	2.0	0.64
	vGLUT1	-0.3	0.93	-2.8	0.15	-1.4	0.46	0.2	0.91	-0.2	0.46
	GLS1	1.8	0.56	-1.1	0.81	-0.9	0.64	0.8	0.82	-1.1	0.64
	GRIA2	2.0	0.56	-3.0	0.15	-2.5	0.14	1.2	0.82	-0.5	0.14
	GRIN1	0.5	0.93	2.6	0.15	0.5	0.71	1.2	0.82	-0.3	0.71
	GAD67	1.7	0.56	0.2	0.96	-0.7	0.64	-0.5	0.82	0.9	0.64
	vGAT	0.3	0.93	0.1	0.96	0.3	0.77	-1.1	0.82	1.6	0.77
	GAT1	-0.5	0.93	-0.3	0.96	-0.3	0.77	-0.7	0.82	2.7	0.77
	GABRG2	0.8	0.91	-0.6	0.87	-0.7	0.64	1.4	0.82	-0.5	0.64
PPC	EAAT2	-0.4	0.93	-0.7	0.87	-0.8	0.64	-0.7	0.82	0.5	0.64
	vGLUT1	2.0	0.56	-1.2	0.70	0.7	0.65	-0.9	0.82	0.3	0.65
	GLS1	1.7	0.56	0.2	0.96	2.8	0.09	-1.0	0.82	-0.3	0.09
	GRIA2	1.1	0.87	-0.3	0.94	0.6	0.65	0.5	0.82	-0.1	0.65
	GRIN1	1.9	0.56	1.4	0.68	1.0	0.64	0.4	0.82	0.6	0.64
	GAD67	1.4	0.83	0.7	0.87	1.8	0.29	-1.0	0.82	0.2	0.29
	vGAT	-0.4	0.93	0.2	0.96	0.8	0.64	-1.9	0.82	2.1	0.64
	GAT1	0.2	0.93	0.1	0.96	0.4	0.77	-1.5	0.82	1.7	0.77
	GABRG2	1.0	0.91	0.9	0.83	3.1	0.09	-0.4	0.82	1.5	0.09
V2	EAAT2	1.1	0.87	1.8	0.38	0.6	0.65	0.2	0.91	1.7	0.65
	vGLUT1	0.6	0.93	0.6	0.87	0.6	0.65	-1.0	0.82	2.6	0.65
	GLS1	0.5	0.93	2.2	0.25	1.1	0.64	-1.5	0.82	0.3	0.64
	GRIA2	0.9	0.91	0.5	0.88	-1.0	0.64	-1.7	0.82	1.1	0.64
	GRIN1	0.8	0.91	0.6	0.87	2.3	0.18	-0.9	0.82	1.7	0.18
	GAD67	0.2	0.93	1.9	0.38	1.6	0.37	-0.9	0.82	1.9	0.37
	vGAT	0.2	0.93	0.5	0.87	2.9	0.09	-0.3	0.83	1.8	0.09
	GAT1	0.5	0.93	1.4	0.68	1.7	0.33	-0.5	0.82	2.7	0.33
	GABRG2	-0.2	0.93	2.4	0.20	1.0	0.64	-0.6	0.82	1.1	0.64
V1	EAAT2	1.1	0.87	-0.1	0.96	0.1	0.90	0.5	0.82	0.7	0.90
	vGLUT1	0.7	0.91	0.4	0.92	0.7	0.64	-0.8	0.82	3.2	0.64
	GLS1	0.7	0.91	0.7	0.87	2.1	0.23	-0.8	0.82	1.1	0.23
	GRIA2	1.7	0.56	1.0	0.81	0.9	0.64	0.1	0.96	1.7	0.64
	GRIN1	-0.1	0.96	3.2	0.15	3.3	0.09	-0.5	0.82	1.6	0.09
	GAD67	1.1	0.87	1.0	0.81	2.0	0.24	-0.4	0.82	1.9	0.24
	vGAT	0.8	0.91	1.3	0.70	2.8	0.09	-0.2	0.91	2.1	0.09
	GAT1	0.1	0.93	0.9	0.83	2.0	0.24	-1.1	0.82	2.4	0.24
	GABRG2	-0.2	0.93	0.8	0.83	1.4	0.44	-0.5	0.82	1.0	0.44

***P values** corrected for multiple comparisons using the Benjamini-Hochberg Method for False Discovery Rate for each comorbid factor

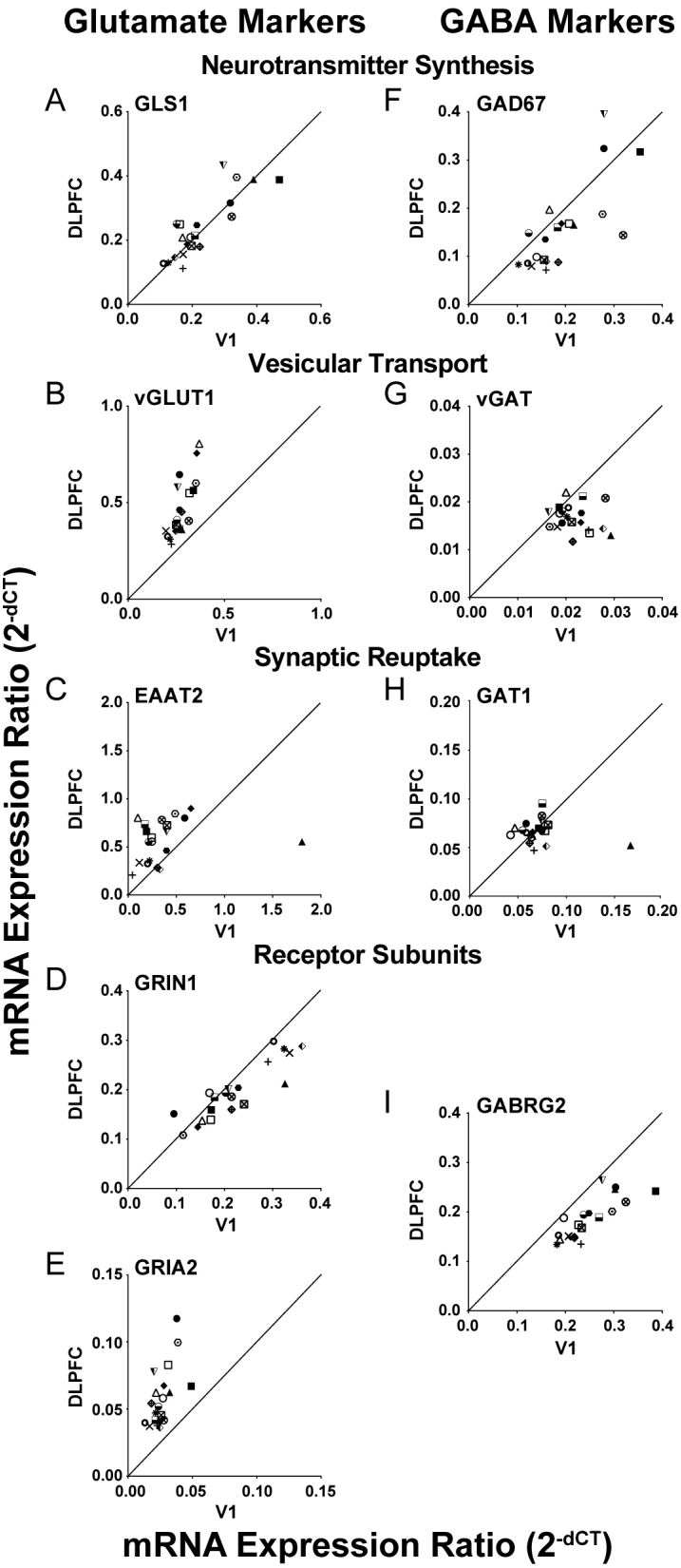
Supplemental Table S4. Adjusted P-values using Benjamini-Hochberg Method for False Discovery Rate.

Diagnosis, Region and Diagnosis-by-Region effects in 20 matched pairs of comparison subjects and subjects with schizophrenia

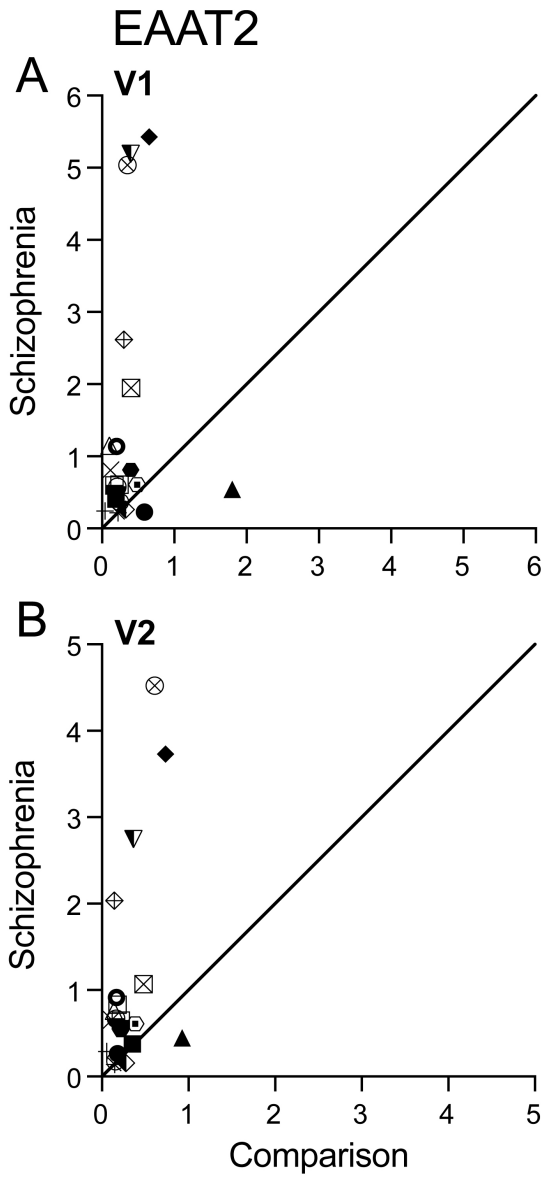
Transcript	Effect	F	P
EAAT2	Diagnosis	$F_{1,114} = 5.6$	*0.04*
	Region	$F_{3,114} = 4.5$	*0.01*
	Diagnosis by Region	$F_{3,114} = 16.0$	< *0.001*
vGLUT1	Diagnosis	$F_{1,114} = 9.3$	*0.008*
	Region	$F_{3,114} = 56.1$	< *0.001*
	Diagnosis by Region	$F_{3,114} = 0.4$	0.85
GLS1	Diagnosis	$F_{1,114} = 0.8$	0.50
	Region	$F_{3,114} = 0.2$	0.92
	Diagnosis by Region	$F_{3,114} = 0.7$	0.63
GRIA2	Diagnosis	$F_{1,114} = 1.2$	0.42
	Region	$F_{3,114} = 93.3$	< *0.001*
	Diagnosis by Region	$F_{3,114} = 0.8$	0.61
GRIN1	Diagnosis	$F_{1,114} = 2.4$	0.21
	Region	$F_{3,114} = 9.9$	< *0.001*
	Diagnosis by Region	$F_{3,114} = 2.3$	0.16
GAD67	Diagnosis	$F_{1,114} = 1.5$	0.37
	Region	$F_{3,114} = 13.4$	< *0.001*
	Diagnosis by Region	$F_{3,114} = 0.6$	0.69
vGAT	Diagnosis	$F_{1,114} = 2.7$	0.19
	Region	$F_{3,114} = 14.3$	< *0.001*
	Diagnosis by Region	$F_{3,114} = 2.8$	0.09
GAT1	Diagnosis	$F_{1,114} = 0.0$	0.98
	Region	$F_{3,114} = 8.0$	*0.0002*
	Diagnosis by Region	$F_{3,114} = 5.9$	*0.003*
GABRG2	Diagnosis	$F_{1,114} = 0.8$	0.50
	Region	$F_{3,114} = 21.7$	< *0.001*
	Diagnosis by Region	$F_{3,114} = 0.7$	0.63



Supplemental Figure S1. Laser capture microdissection approach. A. Representative image of V1 with the laminar boundaries demarcated before microdissection. **B.** The same area of V1 with layer 3 microdissected and captured into the microcentrifuge tube cap. **C.** 5X magnification image of the resulting layer 3 tissue strip captured in the microcentrifuge tube cap. **D.** Representative example of a tube cap with layer 3 strips captured after tissue microdissection from a single subject.



Supplemental Figure S2. Differences between V1 and DLPFC in expression levels of glutamate and GABA marker transcripts in unaffected comparison subjects. Individual subject symbols are the same across region and transcript for all graphs. V1, primary visual cortex; DLPFC, dorsolateral prefrontal cortex.



Supplemental Figure S3. EAAT2 unity plots for V1 and V2. A. Unity plot for V1. **B.** Unity plot for V2. Individual subject pair symbols are the same across region. Mean EAAT2 mRNA levels were higher in V1 (+286%) and V2 (+258%) in schizophrenia. When excluding the subject pairs with mean percent differences > 1000% (3 subject pairs in V1 and 1 subject pair in V2), the mean percent differences were still higher in V1 (+163%) and V2 (+232%). V1 = primary visual cortex; V2 = association visual cortex.

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