Supplementary Materials. Supplementary Methods and Results.

Tissue acquisition and preparation. Subjects were matched for age, postmortem interval (PMI) and pH (Table S1). The medical records of the subjects designated as controls were examined using a formal blinded medical chart review instrument as well as in person interviews with the subjects and/or their caregivers. All studies and data analyses described in this manuscript were performed by technicians that were blind to diagnosis and treatments. The subjects were evaluated for National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINCDS-AIREN) criteria for a diagnosis of vascular dementia; NINCDS, Diagnostic and Statistical Manual of Mental Disorders-IV (DSMIV) and Consortium to Establish a Registry for Alzheimer's Disease (CERAD) diagnosis of dementia; Consensus criteria for a clinical diagnosis of Probable or Possible diffuse Lewy body disease; unified Parkinson's disease rating scale (UPDRS) for Parkinson's disease; clinical criteria for diagnosis of Frontotemporal dementia; medical history of psychiatric disease; history of drug or alcohol abuse; and other tests of cognitive function including the mini mental status exam (MMSE) and clinical dementia rating (CDR). In addition, each brain tissue specimen was examined neuropathologically using systematized macro- and microscopic evaluation using CERAD guidelines. Since the patients in our cohort were elderly at the time of death, many of the subjects have the cognitive impairment associated with aged subjects with schizophrenia [1-4]. All samples were derived from the left side of the brain. Subjects with schizophrenia were diagnosed with this illness for at least 30 years. Samples blocks were processed for Western blot analyses using a dissection scope. Guided by a nissl stained section for each subject, as well as images of the subject generated using in situ hybridization for EAAT2, the mediodorsal (MD) nucleus and the ventral tier nuclei were

dissected with a scalpel.

Laser capture microdissection. The Veritas Microdissection instrument and CapSure Macro LCM caps (Molecular Devices, formerly Arcturus, Mountain View, CA) were used for laser capture microdissection (LCM). Frozen tissue sections were thawed at room temperature and fixed with PALM Liquid CoverGlass N (P.A.L.M. Microlaser Technologies AG, Bernried, Germany) and allowed to dry. Tissue sections were rehydrated with distilled H_20 and then underwent rapid nissl staining with an RNAse-free cresyl-violet solution (1% cresyl violet, 1% glacial acetic acid, pH 4.0). Slides were then dehydrated through serial ethanol washes and soaked in xylene for 10 minutes. Microdissection was performed under the 20X objective lens with laser settings ranging from 70-100mW in power, and 2,000-3,000 µsec in duration. Separate caps were used for each subject and each cell population. Following cell capture, each cap was separately incubated with 50 µl of PicoPure RNA extraction buffer (Molecular Devices) in a 0.5 mL microcentrifuge tube (Applied BioSystems, Foster City, Ca) for 30 min at 42º C. Samples were then centrifuged for 2 min at 800 × g and stored at -80º C.

RNA isolation and reverse transcription. RNA was isolated from the laser capture microdissected neurons using the PicoPure RNA isolation kit (Molecular Devices, Sunnyvale CA) according to the manufacturer's protocol. cDNA was synthesized using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) using 8 µL of total RNA.

cDNA Pre-Amplification. The TaqMan assays or SYBR-Green primer pairs (Table S2) were pooled and diluted with TE buffer (10mM Tris-Cl, 1mM EDTA, pH 8.0) to a final concentration

of 0.2x, and were combined with Taqman Preamp Master Mix (Applied Biosystems) or SYBR-Green Master Mix (Applied Biosystems), and cDNA for the preamplification PCR reaction. The PCR cycles were: 1 cycle of denaturing at 95ºC for 10 min, then 14 cycles of denaturing at 95 º C for 14 sec and annealing at 60ºC for 4 min. Pre-amplified samples were diluted 1:3 with TE buffer and stored at 20ºC until used in real-time PCR assays.

TaqMan Quantitative RT-PCR. RT-qPCR TaqMan gene expression assays (Applied Biosystems) were used to measure expression of 18s (Hs99999901_s1), β-actin (Hs99999903_m1), cyclophillin A (Hs99999904_m1), vesicular glutamate transporter 1 (VGLUT1: Hs01574209_m1), VGLUT2 (Hs00220439_m1), neuron specific enolase (NSE: Hs00157360_m1), glial fibrillary acidic protein (GFAP: Hs00909233_m1), glutamic acid decarboxylase 67 (GAD67: Hs01065893_m1) EAAT1 (Hs00188193_m1), EAAT2 (Hs00188189_m1) and EAAT3 (Hs01060448_m1). Each reaction was performed in duplicate in a 10 uL volume consisting of 8 uL Jumpstart Taq Readymix (Sigma-Aldritch), 0.7 uL TaqMan probe, 3.3 uL RNAse/DNAse free water, and 3 uL pre-amplified cDNA. Cycling conditions included an initial 2-minute hold at 50°C and a 10-minute denaturation at 95°C followed by 50 cycles of 95°C for 15 seconds and 60°C for 1 minute. A pooled calibrator cDNA sample and a set of serial dilutions ranging from 1:5 to 1:40 were included on each plate to determine a standard curve for the quantification of each gene. For the negative controls, cDNA was replaced with an equivalent volume of RNAse/DNAse-free water. Each assay was performed in 96-well optical reaction plates (Stratagene) on a Stratagene Mx3000P qPCR system. The standard curve method was used for relative quantification. The values for duplicate samples were averaged and normalized to the geometric mean of 3 housekeeping genes, 18s, β-actin and cyclophillin A.

SYBR-Green Quantitative RT-PCR. SYBR-Green RT-qPCR primers were designed with the Sci-Ed Central Primer Designer software (Scientific & Educational Software)(Table S2). Each reaction was performed in duplicate in a 20 uL volume consisting of 10 uL SYBR-Green PCR master mix (Applied Biosystems), 5 uL RNAse/DNAse free water, 10 pmol of forward primer, 10 pmol of reverse primer, and 3 uL pre-amplified cDNA. Cycling conditions included an initial 10 minute denaturation at 95°C followed by 60 cycles of 15 seconds at 95°C and 1 minute at the annealing temperature. An annealing temperature of 63°C was used for EAAT2 exon 9-skipping (EAAT2x9) and 59°C for all other assays. The same calibrator sample, negative control, plate set-up and PCR protocols were used as with the TaqMan expression assays. All of our SYBR-Green assays showed a single band at the predicted size; we confirmed the specificity of the assays by excising, extracting, and sequencing the amplified DNA from these validation studies.

RNA integrity studies. 20 μm tissue sections were scraped from glass slides using a sterile scalpel. Total RNA was isolated using the Trizol reagent and protocol. RNA samples were analyzed using protocols and supplies described in Agilent RNA 6000 Nano Kit guide (Cat#5067-1511). RIN values were generated with the Agilent 2100 bioanalyzer (software ver. B.02.07.SI532).

Immunofluorescence studies. We performed qualitative immunofluorescence studies using tissue sections from 4 subjects with schizophrenia and 2 control subjects as previously described [5]. One of these schizophrenia subjects was provided by the Alabama Brain Collection (ABC) (female, age 63 years) that was not included in the other studies in this report. Fresh frozen

thalamic sections (40μm for the ABC sample, 20 μm for all others) were removed from the freezer, air dried for 5 minutes, a circle drawn around each section with a wax pen, and then dried for 5 minutes. Sections were then incubated in phosphate buffered saline (PBS, pH 7.4) for 10 minutes on an orbital shaker. The sections were then fixed with 4% paraformaldehyde solution for 10 minutes at -20°C. Sections were washed again with PBS for 10 minutes, and blocked with 10% normal donkey serum (Jackson Immuno Research, #017-000-001) diluted with PBS for two hour at room temperature. Next, sections were incubated overnight at 4°C in a humidified chamber with the primary antibody (EAAT2, 1:1000, Millipore #AB1783; NeuN, 1:500 dilution, Abcam ab77315). The EAAT2 antibody was tested using cortical brain tissue homogenates and Western blot analysis, and found to give bands that migrated at the expected molecular weight [6-8]. Preadsorption with a blocking peptide for this EAAT2 antibody eliminated the EAAT2 bands on the Western blot [9]. Antibodies were diluted with 0.3% triton X-100 in PBS with 5% normal donkey serum. For our negative isotype control studies, we replaced the primary antibody with species specific IgG at the same dilution. Following incubation with the primary, sections were washed 3 times with PBS for 10 minutes each. Secondary antibodies were diluted in 0.3% Tx PBS with 5% normal donkey serum and incubated with the sections in the dark for two hours at a 1:400 dilution (donkey anti-guinea pig with Alexafluor 488, (A-11073), Life Technologies, and donkey anti-mouse conjugated with Alexafluor 594, (A-21203) Life Technologies and then washed in PBS for 10 minutes at room temperature. To remove background fluorescence, we applied 4 drops of autofluorescence reducer to each section for 5 minutes (Autofluorescence Eliminator Reagent with Sudan Black B, Millipore 2160). Sections were then incubated in 75% ethanol for 5 minutes and washed 3 times with PBS for 10 minutes. Slides were then tapped dry and mounted with 1-2 drops of ProLong

Gold antifade mounting media with DAPI (Invitrogen, P36931). We then viewed the labeled sections using a Leica confocal microscope and generated images using 25 stacks.

Western blot analysis. Western blot studies were performed as previously described [10, 11]. Commercial antibodies for EAAT1 (rabbit anti-EAAT1 (sc-15316) 1:200 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), EAAT2 (guinea pig anti-EAAT2 (AB1783) 1:1000 dilution; Millipore, Billerica, MA, USA), EAAT3 (Rabbit anti-EAAT3 (EAAC11-S) 1:1000 dilution; Alpha Diagnostic International, San Antonio, Texas, USA), G-protein pathway suppressor-1 (GPS1) (Goat anti-GPS1 (ab10413) 1:1000 dilution; Abcam), GAD67 (Mouse anti-GAD67 (MAB5406) 1:1000; Chemicon) and β-tubulin ((MAB1637) 1:10 000 dilution; Upstate). Species appropriate IR-Dye labeled secondary antibodies (1:10 000 dilution; Li-Cor, Lincoln, Nebraska, USA) were used to detect primary antibodies.

Thalamic tissue was dissected from the blocks used for generating tissue sections for our LCM studies. We estimate that there were 3-4 mm of tissue left on each block yielding 500-1000 μl homogenized tissue, with typical concentrations in the 2-6 μg/μl range for most samples. Tissue dissected from specific thalamic nuclei was thawed on ice and homogenized with a polytron at speed setting 5 for 60 seconds in buffer containing 0.32M sucrose, 5mM Tris-HCl, and 1 tablet of EDTA-free Complete Mini protease inhibitor (Roche) per 20 mL total volume. Homogenized brain samples were placed in reducing buffer containing β-mercaptoethanol and heated at 70°C for 10 min. Duplicate samples were run by SDS–polyacrylamide gel electrophoresis on Invitrogen (Carlsbad, California, USA) 4–12% gradient gels, and then transferred to polyvinylidene fluoride membrane using BioRad semi-dry transblotter (Hercules, California, USA). The membranes were blocked in Li-Cor blocking buffer for 30 min at room

temperature, and probed with primary antibody in Li-Cor blocking buffer. Blots were incubated in EAAT2 and EAAT3 antibodies overnight at 4^{o}C while samples were incubated with EAAT1, GAD67, GPS1 and β-tubulin antibodies at room temperature for 2 hours. The membranes were washed three times for 5 min each in 0.01% Tween phosphate buffer solution then probed with IR-Dye labeled secondary antibodies in Li-Cor blocking buffer for 2 hours at room temperature. Washes were repeated after secondary labeling and then placed in water before imaging. The blots were scanned using the Li-Cor laser-based image detection method. We tested our EAAT1, EAAT2, EAAT3, GPS1, GAD67, and beta-tubulin Western blot assays using varying concentrations of total protein of human cortical tissue homogenate. These control studies demonstrated that our assays were linear for the protein concentrations used in our studies.

Animal studies. Rodent studies were performed in accordance with the institutional animal care and use committee (IACUC) guidelines at the University of Alabama at Birmingham. All studies and data analysis were performed by technicians that were blind to treatments. Adult male Sprague-Dawley rats (8 weeks; 250 g) were housed in pairs and maintained on a 12 hour light/dark cycle. Rats were randomized to receive 28.5 mg/kg haloperidol-decanoate or vehicle (sesame oil) by intramuscular injection every 3 weeks for 9 months [12-15]. Brain tissue was dissected and stored at -80^{o}C. Two different sets of rats (N = 10 per group, 40 rats total; sample size determined based on previous studies) were used for the protein and mRNA studies. For the mRNA studies, the right half of each rodent thalamus was processed with the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. cDNA was then reverse transcribed, amplified, and analysed as described above using Sybr-Green RT-qPCR (Table S2). For our protein studies, the right half of each rodent thalamus was thawed on ice and homogenized with

a polytron at speed setting 5 for 60 seconds in buffer containing 0.32M sucrose, 5mM Tris-HCl, and 1 tablet of EDTA-free Complete Mini protease inhibitor (Roche) per 20 mL total volume. Western blots were run and quantified as described above.

Quality control studies.

Since application of the laser capture microdissection (LCM) methodology to human postmortem brain tissue is relatively novel, we performed several quality control studies to demonstrate the feasibility and validity of the technique [16-18]. We first piloted our LCM studies using tissue from a region of convenience, the anterior cingulate cortex. We chose this region based on availability of tissue sections on glass slides, as well as the readily identifiable morphology of the large pyramidal neurons in the deep layers of the cortical gray matter (Figure S1). We found we were consistently able to harvest 500 large cells with pyramidal-neuron morphology and 500 small cells from the same tissue section in about 3 hours. Each of the cell types were harvested on a separate capture cap. Small cells with a small, circular, punctuate nucleus were selected for harvest, and assumed to be a mixture of glia and interneurons. These enriched cell populations were assayed using QPCR for markers of pyramidal neurons (vesicular glutamate transporter1 (VGLUT1) and neuron specific enolase (NSE)), as well as astrocytes (glial fibrillary acidic protein (GFAP)) and interneurons (glutamic acid decarboxylase 67 (GAD67)) as described above. The QPCR cell-marker data (Figure S2) are expressed as a ratio of the values of large cells to small cells for each subject analyzed (N = 5-7); this ratio serves as an internal control for variability between LCM sessions. The large cells are enriched for VGLUT1, while the small cells are enriched for GFAP and GAD 67 (Figure S2). The ratios for NSE were equivocal, consistent with the presence of neurons in both the large and small cell

preparations (Figure S2).

One of the challenges of working with human postmortem tissue is RNA integrity (RIN). Postmortem samples are already at risk for degradation due to the postmortem interval (PMI), which is often more than 10 hours, due to the unavoidable collection processes associated with tissue banking. In addition, the LCM method involves staining the section and keeping it at room temperature for up to 3 hours. Thus, we simulated the LCM conditions, and incubated tissue sections at room temperature for 0 or 3 hours, with or without staining (Figure S3). Tissue sections were then scraped from the glass slide using a sterile scalpel blade, and processed for mRNA extraction, QPCR, and bioanalysis as described above. We found that tissue sections stored for 3 hours at room temperature following staining with our modified RNAse-free nissl stain had RIN values similar to sections processed right out of the freezer (0 hours, no stain)(Figure S3). Staining and 3 hours at room temperature generally did not impact mRNA expression levels, with the possible exception of NSE transcripts.

We were not able to detect a measurable RIN signal with laser-microdissected samples with up to 2,000 cells/sample. However, we have successfully performed microarray analysis (unpublished observation) using mRNA extracted from 2,000 pyramidal neurons using methodology identical to that in this manuscript, and found the sample was suitably intact for high fidelity hybridization to the array substrate. In summary, our quality control studies suggest that we are able to consistently harvest an enriched population of neurons based on morphological identification with minimal impact on mRNA quality.

In situ hybridization. The top panel in figure 1 was generated using [35S]-in situ hybridization as previously described [19].

Power analyses. Power analyses for these experiments were based on means and variances from prior (historical) region level studies for mRNA or protein. For example, based on a detecting a difference of mean protein concentrations of 0.25, a sample standard deviation of 0.25, N = 20 per group, and k = 2 groups, we have calculated a 'phi' statistic of 2.2, which yields an approximate power value (1 – beta) of 0.94.

Additional statistical matters. Because our subject groups have unequal numbers, we assessed each of our dependent measures for compliance with homogeneity of variance using the F test. The large majority of our dependent measures were compliant; three of our dependent measures where we detected changes in schizophrenia versus controls with ANOVA violated this assumption. For these three measures we performed Welch's t-test which probes for statistical differences between groups with unequal variances; two were significant (Taqman assay small cells, EAAT1 P = 0.004, Sybr green assay large cells EAAT2b, P = 0.02), and a third was near significant (Sybr green assay, large cells, EAAT2, P = 0.1).

Supplementary discussion:

In contrast to our findings for the EAAT1-2, we did not detect significant differences in GAD67 or GPS-1 protein expression in the MD or ventral tier nuclei in schizophrenia. GPS-1 is an EAAT2 interacting protein reported to be a negative regulator of EAAT2-mediated glutamate uptake activity [20]. We previously reported increased cortical expression of this protein in schizophrenia, suggesting differential regulation of EAAT2 function in these brain regions. Our GAD67 findings are consistent with a prior study that found no changes in GAD67 mRNA

expression in the MD nucleus, although GAD67 mRNA levels were significantly altered in the anterior nucleus [21, 22]. Since other markers of interneurons have not been comprehensively studied in the thalamus in schizophrenia, the possibility remains there may be subtle changes in GABAergic interneuron function in this illness.

We found an effect of sex on EAAT2 mRNA in small cells using our Sybr green, but not Taqman, LCM-QPCR assay. Our sample cohort were not well balanced for sex (supplementary Table 1), which is a possible limitation of the study. However, we did not detect any other effects of sex with our other dependent measures, raising the possibility that this sex effect for EAAT2 is a false positive. An alternative explanation is that the change seen with the sybr assay is a true positive, and there may be a subtle effect of sex on transporter expression, as estrogen is a potent regulator of EAAT2 expression in astrocyte cultures [23].

Our initial immunofluorescence studies were performed using fresh frozen 20 μm sections. At this thickness, appreciable neuronal density is much lower than in 30-50 μm sections which are typically used for staining. In a subset of our schizophrenia sujects, we primarily found EAAT2 labeling in intermediate size neurons. This raises a concern for our LCM findings as we generally targeted larger relay neurons for capture. To address this concern, we performed additional EAAT2 and NeuN dual label studies using a schizophrenia subject from the Alabama Brain Collection. Using 40 μm sections prepared for immunohistochemical staining, we found colocalization of NeuN with EAAT2 protein in a subset of larger relay neurons in this schizophrenia subject. Overall we observed a higher density of intermediate size neurons, compared to large neurons, that were dual-labeled in this confirmation study. Interestingly, there is well documented heterogeneity of excitatory relay neuron size in the dorsal thalamus; it has been suggested that the smaller relay neurons projects to cortical layers 1 and 3, while the larger

relay neurons project to layers 4 and 6 [24].

Technical considerations. There are a number of technical issues to consider for our study. mRNA levels were measured using a combination of Taqman and Sybr chemistries in this study, due to the mixed availability of splice variant primers. EAAT1 was decreased in small cells following RT-PCR using Taqman assays. No change was found following analysis with Sybr PCR. This is most likely due to the lower sensitivity of Sybr assays with assay design and template quality being potential factors [25].

There are three functionally distinct subregions of the MD nucleus: anteromedial magnocellular, the posterolateral densocellular, and the dorsolateral parvocellular (MD_{PC}) [26]. The cells captured in this study were cut from throughout the MD nucleus and are not representative of any single subregion. The MDpc subregion is possibly the most relevant in the study of schizophrenia due to its reciprocal connections with the ACC and DLPFC which have been implicated in the pathophysiology of schizophrenia [27].

Our large cell population consists of glutamatergic relay neurons, which are larger than astrocytes and interneurons, and have a morphologically characteristic profile for laser capture (Figure 1). In contrast, our small cell population that is enriched for astrocytes likely includes some interneurons, as the morphological profiles of these cells may be similar with nissl staining. We used QPCR and measured expression of neurochemical markers for relay neurons (VGLUT2, Figure 1), pyramidal neurons (VGLUT1, Figure S2), all neurons (NSE, Figure 1 and S2), interneurons (GAD67, Figure S2), and astrocytes (GFAP, Figures 1 and S2). We expressed the data in our neurochemical studies as a ratio of large cells/small cells, with each data point reflecting large and small cells cut from the same slide in the same LCM session. This approach controlled for variability between sessions, providing a quality control measure that we used to

assess the fidelity of cell targeting between LCM sessions. Although low levels of VGLUT transcripts may be expressed in astrocytes, expression levels are markedly higher in excitatory neurons making the use of VGLUTs as neurochemical markers feasible [28]. Finally, neurons are typically enveloped in astrocytic processes, which may contain transcripts for EAAT2 [29]. This raises the possibility that mRNA extracted from our large cell population may have a small amount of astrocyte mRNA. Further work will be needed to examine the relative contributions of mRNA in processes versus excitatory neuron cell bodies.

Effects of nicotine and medications other than antipsychotics. Data on nicotine use for most of our subjects was not available, and thus we were not able to assess the impact of this factor on our dependent measures. While this is a limitation of the study, a large majority of these subjects were institutionalized prior to death for long periods of time, and did not have access to alcohol or drugs of abuse. Finally, there were also not enough subjects on other psychotropic compounds, such as antidepressants, to perform secondary analyses for the effects of these factors.

Rodent antipsychotic studies. We also examined the effects of antipsychotic medications on our dependent measures. EAAT1 protein levels were decreased in the MD nucleus in schizophrenia with a decrease in EAAT1 mRNA levels in a population of cells enriched for astrocytes. In contrast, rats treated chronically with haloperidol had increased levels EAAT1 and EAAT1x9 mRNA and no changes in EAAT1 protein levels in the dorsal thalamus. EAAT2 and EAAT2b mRNA levels were increased in an enriched population of relay neurons in schizophrenia, with no change in expression in the small cell population, while in haloperidol treated rats there was a region-level increase in EAAT2 mRNA, but not EAAT2b, in the thalamus. However, EAAT2

protein levels were decreased in the MD nucleus in schizophrenia, with a large increase in EAAT2 protein levels in antipsychotic treated rats in the dorsal thalamus. Given these generally divergent findings, we posit that the changes we found in schizophrenia are not a result of antipsychotic drug treatment. In addition, the large increase in EAAT2 protein expression with chronic haloperidol treatment suggests that antipsychotic treatment may be correcting a deficit in EAAT2 protein expression in some subjects. Since we did not examine cell-level changes in the thalamus in haloperidol treated rats in the present study, the possibility remains that changes in mRNA and protein expression in schizophrenia are impacted by haloperidol treatment at the cell-level. We have examined cell-level expression of these transcripts in pyramidal neurons in the frontal cortex, and found that nine months haloperidol-decanoate treatment did not affect levels of EAAT2b, EAAT1x9 or EAAT2x9 transcripts in schizophrenia [30].

One limitation of our antipsychotic rat studies is that they were in a normal substrate, while the effects of antipsychotics could be specific to disease states. For the present study we did not have sufficient numbers of schizophrenia subjects off medications at the time of death, or data on lifetime antipsychotic treatment (chlorpromazine equivalents), to perform secondary statistical analyses probing for an effect of antipsychotic medication on our dependent measures. However, previous region level studies found no changes in EAAT expression with haloperidol treatment, while other work found a decrease in EAAT2, but not EAAT1, protein levels following treatment with clozapine [31-36]. In the striatum, EAAT2 mRNA levels and glutamate uptake were decreased following haloperidol treatment [37, 38].

The balance of these studies suggests that our cell-level results in the thalamus are not solely due to a medication effect. We focused on haloperidol in our studies as most of our subjects were taking typical antipsychotics. The previous reports differ from our study in that we

simulated a lifetime of antipsychotic treatment by administering haloperidol-decanoate for 9 months. In contrast, most of the studies described above gave antipsychotics for 9 weeks or less.

Recent evidence suggests a role for canonical signaling pathways in the mechanism of haloperidol's effects on EAAT expression. For example, constitutively activated Akt kinase increased EAAT2, but not EAAT1, expression in cultured astrocytes, and other studies have found changes in Akt expression associated with haloperidol treatment in schizophrenia [39-41].

Figure S1. Light micrographs of laser-capture microdissection of pyramidal neurons from the anterior cingulate cortex. Tissue sections were nissl stained with an RNase-free protocol (A). Targeted neurons (B) were identified based on morphology. Asterisks indicate voids in section where captured cells (D) were removed. Magnification: Panels A, B, and C 20x, panel D, 60x.

Figure S2. QPCR analysis of transcripts from large cells (enriched for pyramidal neurons) and small cells (interneurons and glia) harvested using laser capture microdissection. Data for markers of pyramidal neurons (VLGUT1, NSE), astrocytes (GFAP), and interneurons (GAD67) are expressed as means ± SEM of the ratios of values for each subject for 500 large cells/ 500 small cells (n = 5-7 for each transcript). Large and small cells were captured from the same anterior cingulate cortex tissue section with different caps. Vesicular glutamate transporter 1 (VGLUT1), neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP), glutamic acid decarboxylase-67 (GAD67).

Figure S3. Assessment of the effects of the rapid RNase-free nissl stain and three hours at room temperature on mRNA quality of the tissue section. Tissue sections from the anterior cingulate cortex (N = 5) were left at room temperature for either 0 or 3 hours, with or without staining. 3 hours was used since this approximates the time sections are at room temperature during the

LCM protocol. Tissue was scraped from the slide, the mRNA was isolated with Trizol reagent, and the sample was evaluated by electropherogram and QPCR. Data are expressed as means ± SEM. Vesicular glutamate transporter 1 (VGLUT1), neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP), glutamic acid decarboxylase-67 (GAD67), RNA intergrity (RIN).

Figure S4. Dual-immunofluorescence control studies with isotype and species specific preimmune IgG with labeled secondary antibodies: (A) Alexafluor 488 (green), (C) Alexafluor 549 (red) secondary, and (B) yellow (merged).

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Supplementary Table 1. Subject characteristics

	Comparison group				Schizophrenia			
	QPCR		Western blot		QPCR		Western blot	
Study	Taqman	SYBR	MD	V	Taqman	SYBR	MD	V
N	20	15	16	15	12	9	13	13
Age (years)	74 ± 11	73 ± 11	78 ± 13	76 ± 14	79 ± 11	73 ± 11	77 ± 12	77 ± 12
Sex	11f / 9m	8f / 7m	9f / 7m	7f / 8m	3f / 9m	3f / 6m	6f / 7m	6f / 7m
PMI (h)	9.0 ± 8.5	8.7 ± 8.4	8.9 ± 7.1	8.7 ± 7.0	16 ± 8.5	16 ± 9.7	15 ± 8.2	15 ± 8.2
Tissue pH	6.5 ± 0.2	6.5 ± 0.2	6.5 ± 0.3	6.5 ± 0.3	6.6 ± 0.2	6.5 ± 0.2	6.5 ± 0.4	6.5 ± 0.4
RIN	7.0 ± 1.1	7.3 ± 0.7	n/a	n/a	7.0 ± 1.0	7.3 ± 1.0	n/a	n/a
RIN (range)	4.1-8.4	6.3-8.4	n/a	n/a	5.5-8.9	5.5-8.9	n/a	n/a
Rx status	0/20	0/15	0/16	0/15	6/4/2	5/3/1	10/2/1	10/2/1

Age, PMI, pH and RIN expressed as mean ± standard deviation. Abbreviations: number (N), male (M), female (F), postmortem interval (PMI), hours (h), antipsychotic medication status ((Rx) On / off for more than 6 weeks prior to death / unknown), male (m), female (f), laser capture microdissection (LCM), quantitative PCR (QPCR), RNA intergrity (RIN), not applicable (n/a), Taqman QPCR study (Taqman), SYBR-Green PCR splice variant study (SYBR), mediodorsal nucleus of the thalamus protein study (MD), ventral tier nuclei protein study (V).

Supplementary Table 2: Sybr Green QPCR Primers

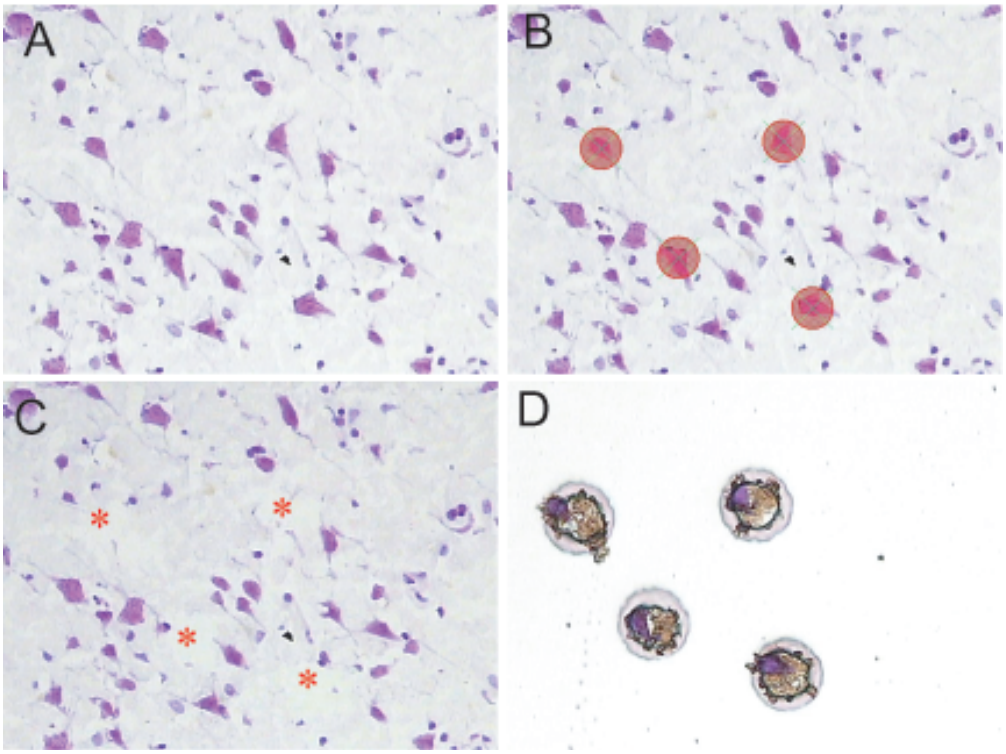
Human Primers

Gene	Forward Primer	Reverse Primer	Accession Number	Amplicon Size
18s	CGCCGCTAGAGGTGAAATTC	TTGGCAAATGCTTTCGCTC	K03432.1	63
Beta Actin	AGTACTCCGTGTGGATCGGC	GCTGATCCACATCTGCTGGA	NM_001101.3	68
Cyclophilin A	CTCCTTTGAGCTGTTTGCAG	CACCACATGCTTGCCATCC	BC137057	324
EAAT1 pan-probe	CGAAGCCATCATGAGACTGGTA	TCCCAGCAATCAGGAAGAGAA	NM_004172.4	489
EAAT1 exon 9 skipping	TGCCCTCTATGAGGCTTTGG	GTCCGGAGGCGATCCCT	NM_004172.4	430
EAAT2 pan-probe	GGGCACCGCTTCCAGTG	ATACTGGCCGCGCCG	NM_004171	124
EAAT2 exon 9 skipping	GGGCACCGCTTCCAGTG	GACTGAAGTTCTCATCCTGTCCCTT	NM_004171	249
EAAT2b	GGACAGGATGAGAACTTCAGTCAA	ACAAGTCTCGATATCCATGAATGG	NM_004171	265

Rat Primers

Gene	Forward Primer	Reverse Primer	Accession Number	Amplicon Size
18s	CGCCGCTAGAGGTGAAATTC	TTGGCAAATGCTTTCGCTC	M11188.1	62
Beta Actin	AGTACTCTGTGTGGATTGGT	GCTGATCCACATCTGCTGGA	NM_031144.2	68
Cyclophilin A	CTCCTTTGAGCTGTTTGCAG	CACCACATGCTTGCCATCC	BC091153.1	323
EAAT1 pan-probe	TGACTGCCGCAGATGCCTTC	TGAGGAGGCCGACGATGACT	NM_019225.1	489
EAAT1 exon 9 skipping	TGCCCTCTATGAGGCTTTGG	GTCCGGAGGCGATCCCT	NM_019225.1	430
EAAT2 pan-probe	CCAGTGCTGGAACTTTGCCT	TAAAGGGCTGTACCATCCAT	NM_017215.2	124
EAAT2 exon 9 skipping	GCTGGGTATCGCCTGCTTGA	CATTCTATCCCTTACAGTCA	NM_017215.2	430
EAAT2b	GGACAGGATGAGAACTTCAGTCAA	ACAAGTCTCGATATCCATGAATGG	NM_017215.2	265

Abbreviations: Excitatory amino acid transporter (EAAT)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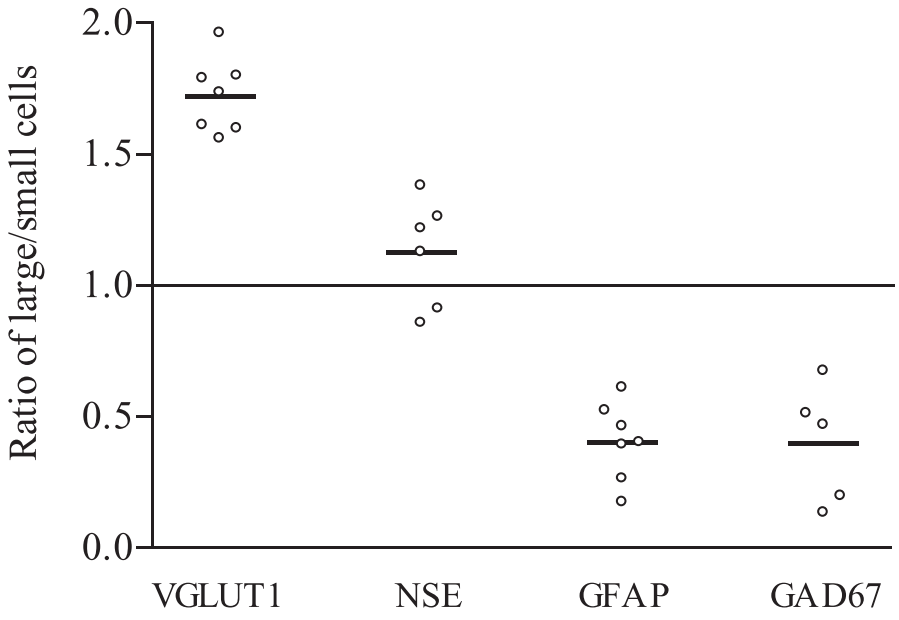
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NSE
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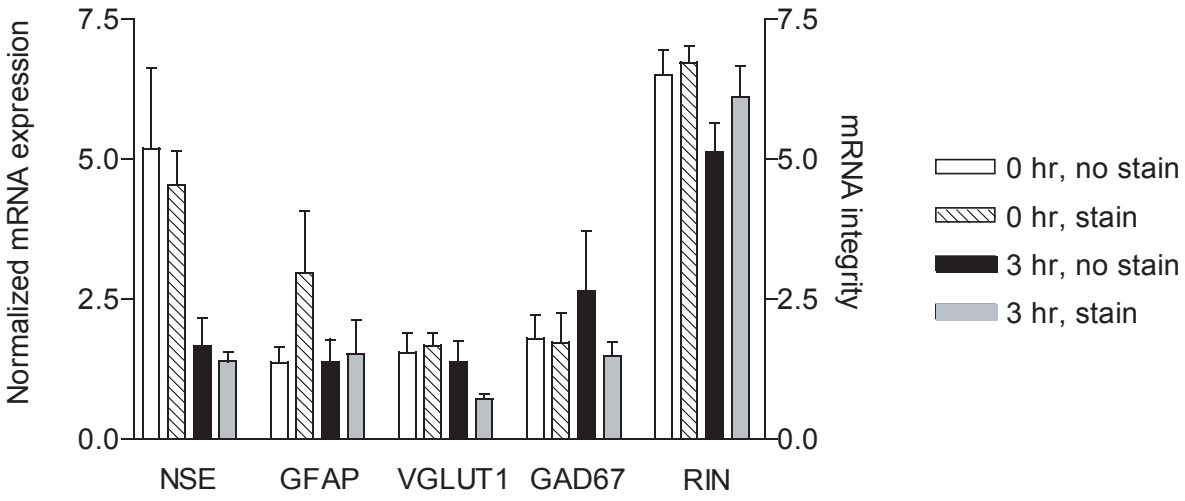
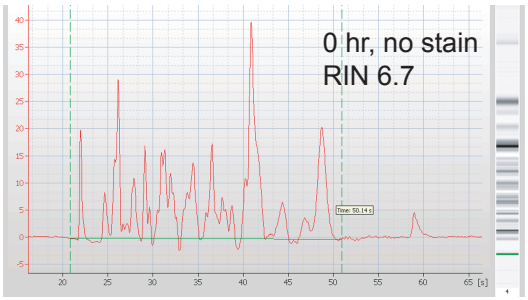
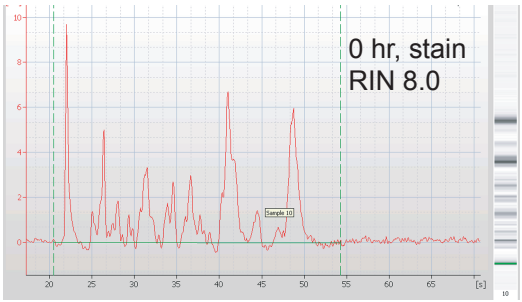
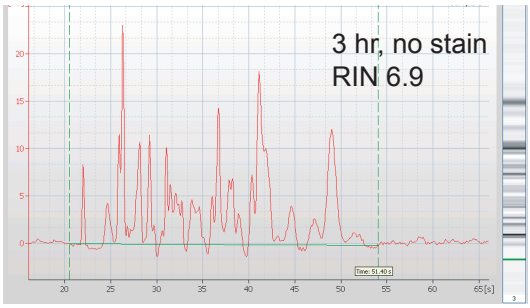
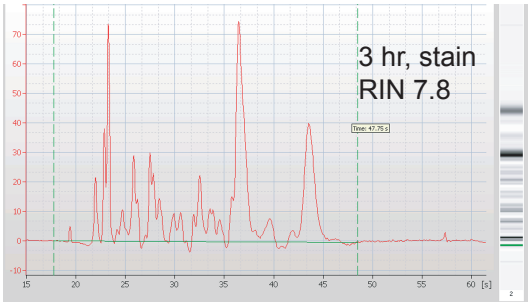


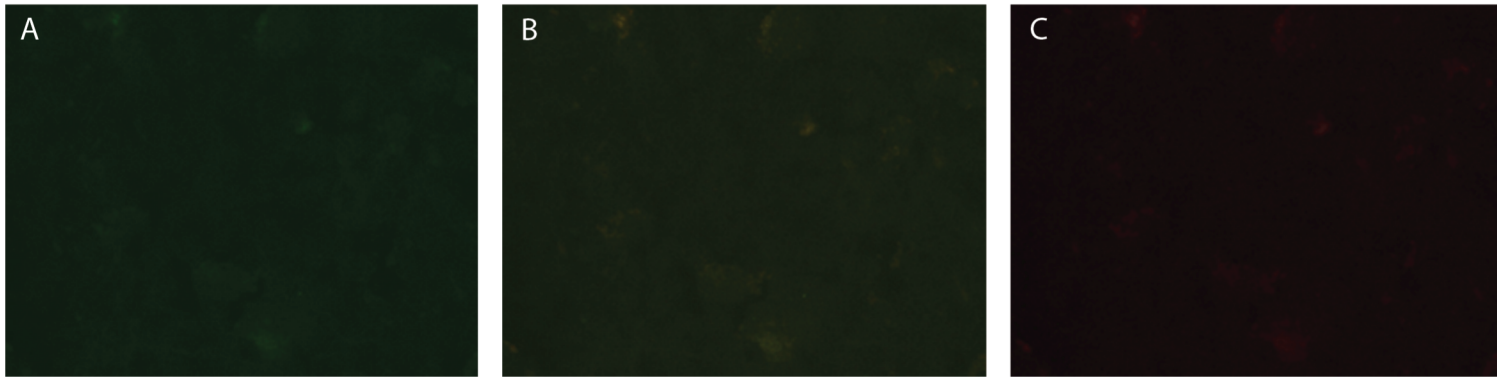
Representative electropherograms







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