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Glutamatergic gene expression is specifically reduced in thalamocortical projecting relay neurons in schizophrenia

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Abstract

Background—Impairment of glutamate neurons which relay sensory and cognitive information from the medial dorsal thalamus to the dorsolateral prefrontal cortex and other cortical regions may contribute to the pathophysiology of schizophrenia. In this study we have assessed the cell-specific expression of glutamatergic transcripts in the medial dorsal thalamus.

Methods and Materials—We used laser-capture microdissection to harvest two populations of medial dorsal thalamic cells, one enriched with glutamatergic relay neurons, and the other with GABAergic neurons and astroglia, from postmortem brains of subjects with schizophrenia (n=14) and a comparison group (n=20). Quantitative polymerase chain reaction (QPCR) of extracted RNA was used to assay gene expression in different cell populations.

Results—The transcripts encoding the ionotropic glutamate receptor subunits NR2D, GluR3, GluR6, GluR7, and the intracellular proteins GRIP1 and SynGAP1 were significantly decreased in relay neurons but not in the mixed glial and interneuron population in schizophrenia.

Discussion—Our data suggest that reduced ionotropic glutamatergic expression occurs selectively in neurons giving rise to the cortical projections of the medial dorsal thalamus in schizophrenia, rather than in thalamic cells which function locally. Our findings indicate that glutamatergic innervation is dysfunctional in the circuitry between the medial dorsal thalamus and cortex.

Keywords

Postmortem gene expression; receptor; laser-capture microdissection; medial dorsal thalamus

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Introduction

Schizophrenia is a debilitating psychiatric disorder which occurs in approximately 1% of the population worldwide. Its etiology is not known, but with accumulating data, the glutamate hypothesis of schizophrenia has gained credibility (1–5). This hypothesis proposes that dysregulation within the glutamate neurotransmitter system contributes to the pathophysiology of schizophrenia, and arose from observations that some drugs blocking glutamatergic receptors can produce a psychosis more closely resembling schizophrenia than psychotomimetic drugs acting on other neurotransmitter systems(6–8). In addition, these drugs can exacerbate symptoms experienced by schizophrenia patients(9). Brain imaging studies reveal reduced glutamate in schizophrenia patients using magnetic resonance spectroscopy imaging (10–12). These findings have been consolidated by observations in postmortem brain, where specific changes in glutamate receptor expression and subunit composition have been detected in multiple brain regions of schizophrenia patients (13–41).

Glutamate is the primary excitatory neurotransmitter in the central nervous system within which glutamatergic neurons form a widely distributed network. Glutamate exerts its actions through ionotropic receptors which are classified as NMDA, AMPA and kainate subtypes, in addition to metabotropic receptors(42). Glutamatergic synapses also contain a distinctive web of proteins beneath their postsynaptic membranes, called the postsynaptic density (PSD), which interacts with the receptors, facilitating glutamatergic transmission. The dynamic rearrangement of dendritic spines and PSDs appears to be the structural basis for synaptic regulation and synaptic plasticity(43). Therefore, the regulation and functional interplay between PSD components leading to the alteration of synaptic structures makes these strong candidates for investigation in disorders associated with cognitive dysfunction, including schizophrenia. Proteins within the PSD include SynGAP, PSD-95, STARGAZIN, SAP97, SAP102, PICK1, GRIP1 and NF-L(44).

Abnormalities within the glutamatergic system have been extensively studied in schizophrenia patients (13–41, 45–47). Several prefrontal cortical regions are considered to be impaired in schizophrenia, including the dorsolateral prefrontal cortex (DLPFC) and the cingulate cortex(48). The thalamus is a critical subcortical structure linked to the cortex. While much of the pathophysiology of schizophrenia has been attributed to cortical dysfunction, disruption of reciprocal neuronal projections between the medial dorsal thalamus (MD) and frontal cortical areas is likely to impair the transmission of information between these two brain regions. Neuronal projections originating in the MD which terminate in the DLPFC and cingulate cortices are glutamatergic relay neurons. These cortical areas send projections to the caudate nucleus and nucleus accumbens, respectively(49).

Thalamic abnormalities have been identified in schizophrenia by multiple brain imaging studies. Reduced thalamic volume has been reported by several investigators, in addition to reduced metabolism in the thalamus (50–52). Thalamic hypofunction is thought to contribute to deficits of sensory processing in schizophrenia patients (53). Some stereological studies of postmortem brain have demonstrated a substantial loss of the neurons in the MD in schizophrenia but there have been conflicting data. It is estimated that 70% of these neurons are glutamatergic, suggesting that some glutamatergic neurons are included in the neuronal loss observed, which might be predicted to disrupt communication between the thalamus and the prefrontal cortex(48).

Studies of thalamic glutamatergic gene expression in schizophrenia have produced mixed results (54–62). However, investigations of whole thalamus and thalamic nuclei using *in situ*

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hybridization, semi-quantitative real time PCR (PCR), western blotting and receptor autoradiography have together suggested a reduction of glutamatergic gene expression in various thalamic nuclei, including the MD of schizophrenia patients(22, 24, 33, 63). Conflicting data between these studies may not be due to differences in measurements of protein and transcript, but rather due to cellular heterogeneity of the tissue tested.

In the current study, we have tested the expression of 35 genes of the glutamate system relative to several 'housekeeping' genes, specifically in a population of medial dorsal thalamic cells enriched for glutamatergic relay neurons compared to a population of smaller cells enriched for GABAergic neurons and astroglia, which were isolated using laser capture microdissection. Cell populations were identified using gene expression markers. We report reductions of a subset of glutamate associated transcripts in schizophrenia patients, which were restricted to the glutamatergic relay neurons of the MD and not in the smaller cell population enriched with GABAergic neurons and astroglia.

Methods

Subjects

Frozen postmortem brain tissue from the thalamus was obtained from two groups of subjects: (1) patients diagnosed by DSM-IV criteria (64) with schizophrenia (SCZ), but no other psychiatric co-morbidities; (2) a comparison group (NC) with no history of psychiatric or neurological disorders. Subjects were recruited by the Mount Sinai/Bronx Veterans Administration (VA) Medical Center Department of Psychiatry Brain Bank. All patients had thorough neuropathologic characterization to rule out neurodegenerative disorders including Alzheimer's disease (65). All subjects died of natural causes and without a history of alcoholism and/or substance abuse. The groups were matched for age, postmortem interval (PMI), tissue pH and sex, as shown in Table S1 in the Supplement. A total of 14 SCZ and 20 NC subjects were tested and not all genes were measurable in each subject. There was adequate statistical power to detect a difference between our subject groups with an effect size of 0.8 (www.danielsoper.com/statcalc). All assessment, consent and postmortem procedures were conducted as required by the Institutional Review Boards of Pilgrim Psychiatric Center, Mount Sinai School of Medicine and the Bronx VA Medical Center.

Harvesting of cells and isolation of RNA from the medial dorsal thalamus

Brains were obtained after autopsy and cut into 0.8–1 cm coronal slabs and stored at − 80°C. Blocks including the medial dorsal nucleus of the thalamus from the left side of the brain of each subject were used in this study. To identify the thalamic nuclei, we used anatomical landmarks by comparison with adjacent *in situ* hybridization images from previous studies (Figure 1B)(63, 66–67), descriptions of thalamic architecture (68), and matched to brain atlases (69). Four adjacent 20 μm sections including the medial dorsal nucleus, were mounted onto 1 × 3 inch penfoil polymer (PEN) or superfrost plus glass microdissection slides and stored at − 80°C before cell harvesting. Sections were incubated in 1% cresyl violet acetate for 2 minutes, submerged in 95% ethanol, then 100% ethanol for 30 seconds, followed by immersion in xylene for 5 minutes. This staining revealed large glutamatergic relay neurons in the medial dorsal thalamus (Figure 1A) with predicted gene expression profiles of neuronal and glial markers (Table 1, Figure 1C). 500–600 of these cells were isolated from each subject by laser capture microdissection using an Arcturus VERITAS instrument and protocols (http://www.moleculardevices.com/pages/instruments/veritas.html). 500–600 smaller cells, with morphology and gene expression patterns consistent with GABAergic neurons and astroglia, were also harvested (Figure 1). Investigation of the mixed small cell population was performed for the purposes of comparison. These cells were controls, because they were

harvested using the same methodology as the large cells, and provided a more accurate control group than a tissue scrape. Total RNA was extracted using the PicoPure RNA isolation Kit (Molecular Devices, Sunnyvale, CA). RNA quality was evaluated using the Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA). Extraction procedures reliably produced average RNA integrity numbers (RIN) ≥7 (Table S1 in the Supplement). 'Test slides' simulated the effects of the LCM process including Nissl staining and a time delay of 3 hours at room temperature before RNA integrity (RIN) was measured (70). All section/cells harvested using LCM were processed in the same manner; thus differences between the diagnostic groups are unlikely to be secondary to a processing effect. We have included RIN measurements for each of the samples in Table S1 in the Supplement.

cDNA was synthesized from equal quantities of RNA from each subject, by the reverse transcriptase (RT) reaction using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Aliquots of cDNA from each subject were pooled for use as standards, with dilutions of 1:20, 1:10, 1:5 and undiluted pooled cDNA used to generate a calibration curve according to the relative standard curve method for gene expression analysis (www.AppliedBiosystems.com).

Preamplification of cDNA from laser captured cells

A preamplification step was required due to the low starting concentration of mRNA (diluted to a final concentration of 20 ng per microliter) after RNA was extracted (71) from 500–600 laser capture micro-dissected cells per subject using the Arcturus protocol. All TaqMan assays intended for gene expression analysis were combined in equal volumes to a final volume of 12.5 μl so that each assay was diluted 1 in 10 in TE buffer. This mixture was added to 12.5 μl of cDNA and 25 μl of TaqMan Preamp Master Mix (Applied Biosystems). Samples were centrifuged and amplified in a thermal cycler (BioRad) for 1 cycle of 95°C for 10 minutes, and then 14 cycles of 95°C for 15 seconds and 60°C for 4 minutes.

Gene expression assays

Primers and probes used were standard proprietary assays designed by Applied Biosystems, for which detailed information can be found at their website: https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=ABGEKeywordSearch&catID=601267 and are listed in Table S2 in the Supplement. TaqMan PCR assays for each target gene were performed in duplicate on cDNA samples in 96-well optical plates on a Stratagene MX3000P (Stratagene, La Jolla, CA). All TaqMan PCR data were captured using Sequence Detector Software (SDS version 1.6; PE Applied Biosystems). For every sample, an amplification plot was generated, showing the increase in the reporter dye fluorescence with each cycle of PCR. ACTB, B2M and GAPDH were stably expressed in the samples tested and were used as standards with which we calculated ratios of gene expression for each test transcript. The geometric mean of their expression levels was used for normalization, since averaging of multiple internal control genes has greater accuracy than calculations from single housekeeping genes(72). The relative expression levels of test transcripts were calculated using the Relative Standard Curve Method according to Applied Biosystems instructions (Guide to Performing Relative Quantitation of Gene Expression Using Real-time Quantitative PCR, Applied Biosystems). Standard curves were generated for each target assay and for each endogenous control assay using a 'calibration curve'. The curve was generated from measurements of a mixture of serial dilutions of a pool of all experimental samples. Using the linear equations of standard curves, the amounts of each mRNA were calculated. The relative expression level of a target mRNA was computed as the ratio of the target mRNA amount to the geometric mean of the amounts of the three endogenous controls.

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Statistical analysis

Our hypothesis was that glutamatergic dysfunction occurs in schizophrenia, and to test this we conducted a two-way ANCOVA, grouping data by diagnosis and candidate gene (SPSS v 18, SPSS Inc, Chicago, IL). *Post-hoc* tests were primarily Student's t test and Levene's test for equality of variances. Where variances were significantly different between cases and controls, the t value reported did not assume equal variances. Age, postmortem interval (PMI) and pH were considered as potential covariates. Comparisons made were the expression level of each gene, with diagnosis as a classification variable. Age at death, PMI and pH were included as covariates if correlated with gene expression using Pearson's correlation coefficient.

Results

Glutamatergic relay neurons were enriched in the large cell population. High levels of vesicular glutamate transporter 2 (VGLUT2) were detected in the large cell populations, but not in the small cell populations sampled from each subject. There were much lower levels of GFAP and GAD-67 in the large cell population in contrast with the small cell population from each subject (Table 1, Figure 1C). High expression levels of VGLUT2 are specific to glutamatergic neurons (73–74), while GFAP and GAD-67 are specific to astroglia and GABAergic cells respectively (75–76). Neuron specific enolase (NSE) expression is expected in all neurons (77). Therefore our finding of higher levels of NSE in the large cell population in comparison with the small cell population, suggests that the small cell population is a mixture of both GABAergic cells and astroglia. These data are summarized in Table 1 and Figure 1C.

Having confirmed that the large cell population was indeed enriched with glutamate neurons, and that the small cell population was enriched with astrocytes and GABAergic neurons, we measured the expression of genes associated with glutamatergic neurotransmission in these two cell populations.

Our hypothesis was that glutamatergic dysfunction occurs in schizophrenia, and to test this we conducted a two-way ANCOVA, grouping data by diagnosis and candidate gene, and our results indicate that there is an overall reduction in glutamatergic transcript level in the large cell population in the schizophrenia cases relative to the comparison group ($F=18.05$, $df=1,64$, $p=0.00003$). In contrast, there were no significant differences in glutamatergic transcript level between the diagnostic groups in the small cell population.

Data from *post-hoc* tests are summarized in Table 2. Our measurements of transcript abundance in the large cell population indicated an overall reduction of all ionotropic glutamate receptor subunits and postsynaptic density proteins, but not metabotropic glutamate receptors, in schizophrenia subjects relative to the comparison group (illustrated in Figure 2). Reductions reaching statistical significance ($p \leq 0.05$) were detected for GluR3, GluR6, GluR7, NR2D, GRIP1 and SynGAP1. In contrast, these transcripts were not found to differ in the small cell population. RIN, pH and other demographic variables (Table S1 in the Supplement) were not associated with diagnosis or gene expression. No significant differences were observed between the genders in any of the variables tested, and therefore we considered it appropriate to combine the males and females in each diagnostic group. There were slightly more subjects in the comparison group (18–20) than in the patient group (12–14) but we retained approximately 71% statistical power in the study to detect large effect sizes (0.8).

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Discussion

This study demonstrates that reduced glutamatergic gene expression occurs specifically in the relay neurons of the medial dorsal thalamus in schizophrenia. When individual genes are considered, our data show (1) significant reductions in GluR3 (an AMPA receptor subunit), GluR6 and GluR7 (kainate receptor subunits), NR2D (an NMDA receptor subunit), GRIP1 and SynGAP transcripts (PSD proteins) in thalamocortical relay neurons in schizophrenia; (2) no alteration of mGluR expression in either cell population; and (3) enrichment of specific cell populations can be performed successfully in the thalamus using morphological features combined with gene expression analysis of neuronal and glial markers combined with laser capture microscopy.

Our findings of reduced glutamatergic gene expression in thalamocortical neurons suggest that the circuitry required for communication between the thalamus and frontal cortex is dysfunctional in schizophrenia. These effects were not observed in the astroglia and GABAergic cells which remain local to the thalamus, suggesting that the deficiency is specific to thalamocortical circuitry and not due to a general defect of all cells of the thalamus.

The glutamate hypothesis of schizophrenia proposes that altered glutamatergic activity may underlie the pathophysiology of the disorder, which originated from observations of schizophreniform psychosis on administration of non-competitive NMDA receptor antagonists such as phencyclidine to normal subjects(6). Many other observations have subsequently supported the hypothesis that glutamatergic hypofunction is psychotomimetic, including the current data, which indicate that a reduction of glutamatergic gene expression occurs in the MD in schizophrenia. Our experiments revealed that proteins involved in ionotropic glutamatergic transmission had the greatest reductions in transcript levels in schizophrenia. This finding was specific to the glutamate neurons of the MD, and not in the astroglia and small GABAergic neurons. These data indicate that cell-specific changes occur in glutamatergic transmission in the medial dorsal thalamus in schizophrenia. These cell-specific effects may explain discrepancies with a previously published study of whole thalamic nuclei where no differences were detected between schizophrenia cases relative to a comparison group (61).

The ionotropic glutamate receptors interact physiologically and therefore our findings of reduced expression of AMPA and kainate receptor subunits in schizophrenia relative to the comparison group is not surprising, because a primary deficit in either NMDA or AMPA function could lead to a secondary downregulation of other glutamate receptors. Activation of AMPARs is necessary to remove the magnesium ion blockade of the NMDAR ion channel, which precedes NMDAR activation and subsequent depolarization of the postsynaptic neurons(86). Reduced expression of AMPAR subunits, leading to reduced AMPAR activity, may lead to altered glutamatergic transmission which has been shown to produce psychosis. Our *post-hoc* findings revealed reductions of both GluR3 and an AMPAR-associated PSD protein, GRIP1 in schizophrenia.

Our *post-hoc* findings indicate that several ionotropic receptor subunit transcripts were reduced in schizophrenia in addition to reduced SynGAP expression. SynGAP is a PSD protein associated with NMDAR signal transduction which is interesting because SynGAP mutant mice have non-habituating and persistent hyperactivity, enhanced startle reactivity, impaired sensorimotor gating, altered social behavior, in addition to deficits in cued fear conditioning and working memory which are similar to other mouse models of schizophrenia(78). Similar abnormal behaviors which could model some features of schizophrenia have been observed in mice engineered with reduced NMDAR function(79–

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82). Our data also indicate reduced expression of NR2D subunits of NMDARs, which are thought to confer greatest activity to the NMDAR complex (84–85). Our gene expression data are generally in concordance with studies showing reduced NMDAR protein levels inferred from receptor autoradiography. However, our cell-specific findings for reduced NR2D expression in schizophrenia have not been previously reported in studies using *in situ* hybridization (22, 63) or semi-quantitative PCR(61) which may be due to differing sensitivities of the methods employed. Our data support the large body of converging evidence that schizophrenia could be associated with abnormalities of NMDAR signal transduction. Our *post-hoc* analyses also indicate reduced expression of the kainate receptor subunits GluR6 and GluR7. These reductions could be secondary to NMDAR deficits or vice versa. Chronic administration of phencyclidine, or PCP, which blocks the NMDAR ion channel and has been shown to induce psychosis(87), also appears to decrease GluR2, GluR3, GluR5 and GluR6 expression in rat prefrontal cortex(88), suggesting that a primary reduction of NMDAR signaling can cause secondary reductions in the expression levels of non-NMDAR subunits.

Reduced fast excitatory transmission in the thalamocortical circuitry between the MD and prefrontal cortex could be the consequence of reduced ionotropic glutamatergic gene expression in the relay neurons from the MD. These relay neurons project to the prefrontal cortex, a region which is considered to be impaired in schizophrenia, leading to deficits in executive function (48). Our data indicate that slow transmission from metabotropic glutamate receptors is less likely to be involved since levels of mGluR transcripts were unchanged in these cells in schizophrenia.

Our data show gene expression differences between cell populations, which may obscure measurements of transcriptional variation in regional studies of gene expression using tissue homogenates, whole nuclei or sections. We report reduced levels of several glutamate receptor transcripts and PSD proteins, specifically in large glutamate cells of the medial dorsal thalamus and not in other cells. The other cells tested ('small cells') comprised a mixed population of astroglia and GABAergic interneurons. While this is an extensive study and provides gene expression data with anatomical specificity, there remain some limitations of this approach. As ever with postmortem studies of schizophrenia, there may be confounding effects due to antipsychotic treatment received by the patients and not the controls. Further, we used assays to measure transcript abundance which did not take into account post-transcriptional variation and therefore isoform differences between the proteins investigated. Measurements of transcript alone may infer gene expression changes in schizophrenia, but mechanistically measurements of protein abundance are required to confirm that protein activity is likely to be reduced. Measuring protein levels in LCM-derived tissue is currently not possible in small anatomical regions due to limitations in tissue quantity. We estimate that 10,000 cells would be required to measure glutamate receptor proteins in these subjects by conventional methods, because proteins cannot be amplified before measurement.

In summary, we have found cell-specific reductions in glutamate receptor transcripts in the MD of schizophrenia subjects relative to a comparison group, specifically in a subset of NMDA, AMPA and kainate receptor subunits, and the PSD proteins SynGAP and GRIP1. These data indicate that dysfunction of glutamate neurotransmission occurs specifically within the neurons giving rise to the projections of the MD to the frontal cortex in schizophrenia, rather than in astroglia or GABAergic neurons, which remain intrinsically local to, and do not send projections from, the thalamus. Thus we suggest that these data reflect an abnormality in schizophrenia of thalamocortical circuitry rather than a global defect of all the cells in the thalamus. Further investigation of protein abundance, trafficking

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and post-transcriptional variation of the candidate genes associated with schizophrenia is required to clarify these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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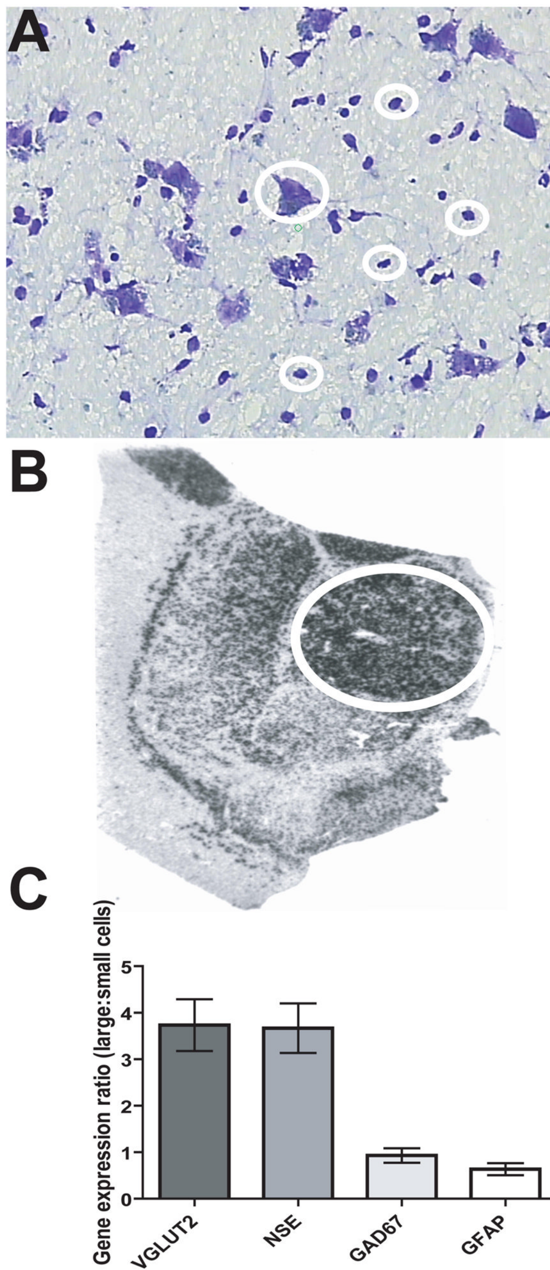

Figure 1. Gene expression studies of human postmortem thalamus

(A) Nissl stained slides of frozen postmortem tissue distinctly reveal large glutamatergic cells consistent with relay neurons in this region. An example of a large cell is encircled in the center of the photomicrograph, and four small cells are also indicated. The latter were a mixture of GABA interneurons and astroglia, which were harvested separately. (B) An autoradiographic image of a section of thalamus labeled with a radioactive riboprobe specific for GAD67 transcript. The medial dorsal (encircled) and lateral and ventral thalamic regions are distinct. (C) The expression of VGLUT2, NSE, GFAP and GAD67 transcripts in large cell sample relative to small cells. Large/small cell expression levels of VGLUT2 was >1, as expected, because VGLUT2 is only expressed in glutamatergic cells. In addition, GAD67 and GFAP were mostly absent in large cells, while NSE was similarly expressed in large and small cells captured. These data indicate that the large cells are enriched for

glutamatergic relay neurons and the small cell population is enriched for GABAergic neurons and astrocytes.

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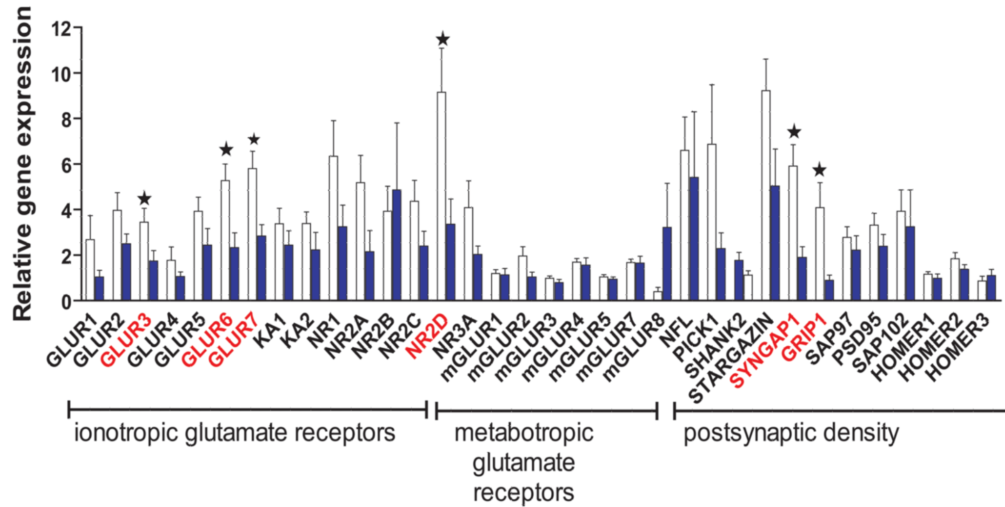

Figure 2. Gene expression in glutamatergic relay neurons of the medial dorsal thalamus in schizophrenia patients relative to comparison subjects

Mean gene expression (ratio of measured transcript level to the geometric mean of the measurements for three housekeeping genes) is plotted for each glutamatergic candidate gene within each subject group. The white bars represent the comparison group and shaded bars represent the schizophrenia subjects. Asterisks indicate statistically significant differences ($p<0.05$) in transcript abundance between schizophrenia subjects and the comparison group.

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Table 1

Characterization of cell populations using neuronal markers.

NEURONAL MARKERS		VGLUT2		NSE		GAD67		GFAP	
	CELL POPULATION	LARGE	SMALL	LARGE	SMALL	LARGE	SMALL	LARGE	SMALL
COMPARISON GROUP	Mean	5.70	1.24	6.53	0.23	0.38	1.11	0.02	2.28
	SEM	1.07	0.27	1.61	0.07	0.19	0.33	0.01	0.46
SCHIZOPHRENIA CASES	Mean	5.82	0.96	4.44	0.14	1.02	0.57	0.03	1.37
	SEM	2.01	0.30	1.41	0.06	0.28	0.25	0.02	0.31
TOTAL	Mean	5.75	1.12	5.61	0.19	0.66	0.87	0.02	1.88
	SEM	1.04	0.20	1.09	0.04	0.17	0.22	0.01	0.30

Large and small cells were isolated in two groups for each subject (schizophrenia cases n=14, comparison group n=20), and the expression of the glutamate neuron marker (VGLUT2), neuron-specific enolase (NSE), the GABAergic cell marker, GAD67, and the astrocytic marker, GFAP, were measured in each sample relative to 3 housekeeping genes (see Methods). The results indicated that the large cell population was enriched for glutamatergic relay neurons, while the small cell population was enriched with GABAergic cells and astroglia.

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TABLE 2

Analyses of transcript abundance in medial dorsal thalamus cell populations of schizophrenia cases and the comparison group.

			LARGE CELLS					SMALL CELLS	
	diagnosis	**N**	**MEAN**	**SEM**	**t**	**df**	**p**	**MEAN**	**SEM**
GLUR1	NC	19	2.68	1.06	1.25	30	0.22	1.34	0.64
	SCZ	13	1.04	0.29				1.12	0.96
GLUR2	NC	19	3.97	0.77	1.47	30	0.15	1.68	0.53
	SCZ	13	2.50	0.43				1.27	0.30
GLUR3	NC	19	3.45	0.60	2.08	30	**0.046**	1.17	0.24
	SCZ	13	1.74	0.46				0.76	0.26
GLUR4	NC	19	1.77	0.59	0.96	30	0.34	0.24	0.05
	SCZ	13	1.06	0.20				0.18	0.04
GLUR5	NC	19	3.93	0.61	1.56	30	0.13	1.22	0.24
	SCZ	13	2.44	0.73				0.67	0.24
GLUR6	NC	19	5.27	0.73	2.83	30	**0.0083**	2.05	0.85
	SCZ	13	2.33	0.65				1.37	0.75
GLUR7	NC	19	5.80	0.76	2.93	30	**0.0064**	0.64	0.19
	SCZ	13	2.84	0.50				0.50	0.17
KA1	NC	19	3.38	0.67	0.97	30	0.34	1.48	0.37
	SCZ	13	2.44	0.63				1.05	0.33
KA2	NC	19	3.39	0.51	1.31	30	0.20	1.80	0.32
	SCZ	13	2.23	0.77				11.95	11.26
NR1	NC	19	6.34	1.57	1.50	30	0.15	1.98	0.62
	SCZ	13	3.25	0.94				0.85	0.17
NR2A	NC	19	5.18	1.20	1.85	30	0.075	1.15	0.64
	SCZ	13	2.15	0.93				0.55	0.22
NR2B	NC	19	3.93	1.09	−0.34	30	0.74	0.71	0.32
	SCZ	13	4.86	2.95				0.91	0.62
NR2C	NC	19	4.36	0.92	1.75	29.4	0.091	1.47	0.23
	SCZ	13	2.40	0.65				1.02	0.20
NR2D	NC	19	9.15	1.94	2.60	27.2	**0.015**	2.28	1.16

	diagnosis	N	LARGE CELLS MEAN	SEM	t	df	p	SMALL CELLS MEAN	SEM
	SCZ	13	3.36	1.10				1.28	0.61
NR3A	NC	19	4.08	1.18	1.66	21.4	0.11	1.27	0.31
	SCZ	13	2.03	0.37				1.14	0.34
mGluR1	NC	20	1.19	0.17	0.18	30	0.86	0.80	0.16
	SCZ	12	1.13	0.28				0.64	0.46
mGluR2	NC	20	1.96	0.41	1.99	27.1	0.057	0.39	0.08
	SCZ	12	1.04	0.21				0.49	0.13
mGluR3	NC	20	0.98	0.10	1.14	30	0.26	1.08	0.34
	SCZ	12	0.79	0.14				0.91	0.22
mGluR4	NC	20	1.69	0.16	0.41	30	0.69	0.44	0.06
	SCZ	12	1.56	0.32				0.76	0.32
mGluR5	NC	20	1.04	0.10	0.67	30	0.51	1.11	0.23
	SCZ	12	0.94	0.10				0.65	0.24
mGluR7	NC	20	1.67	0.15	0.07	16.2	0.95	1.90	0.27
	SCZ	12	1.65	0.30				1.15	0.23
mGluR8	NC	20	0.39	0.19	−1.46	11.2	0.17	.	.
	SCZ	12	3.22	1.93				.	.
NFL	NC	19	6.60	1.46	0.40	30	0.69	2.17	0.43
	SCZ	13	5.41	2.88				1.35	0.51
PICK1	NC	19	6.87	2.61	1.42	30	0.17	1.24	0.34
	SCZ	13	2.29	0.69				0.87	0.27
SHANK2	NC	20	1.77	0.35	1.37	30	0.18	0.98	0.12
	SCZ	12	1.12	0.19				1.02	0.26
STARGAZIN	NC	19	9.22	1.39	1.95	30	0.06	3.23	0.47
	SCZ	13	5.03	1.63				2.25	0.62
SYNGAP1	NC	19	5.91	0.94	3.81	25.8	**0.00077**	1.23	0.25
	SCZ	13	1.90	0.47				1.88	1.00
SAP97	NC	19	2.78	0.47	0.72	30	0.48	0.66	0.20
	SCZ	13	2.22	0.63				0.40	0.08
SAP102	NC	19	3.93	0.93	0.39	30	0.70	1.05	0.28

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			LARGE CELLS					SMALL CELLS	
	diagnosis	N	MEAN	SEM	t	df	p	MEAN	SEM
	SCZ	13	3.25	1.61				1.29	0.92
PSD95	NC	19	3.32	0.52	1.22	30	0.23	0.35	0.07
	SCZ	13	2.39	0.52				0.37	0.18
GRIP1	NC	19	4.08	1.10	2.84	19.6	**0.01**	9.35	7.86
	SCZ	13	0.89	0.23				0.52	0.17
HOMER1	NC	20	1.16	0.11	0.88	30	0.38	1.22	0.15
	SCZ	12	0.98	0.19				1.21	0.25
HOMER2	NC	20	1.84	0.27	1.21	30	0.24	0.98	0.11
	SCZ	12	1.38	0.20				1.19	0.28
HOMER3	NC	20	0.86	0.21	−0.69	30	0.49	2.08	0.78
	SCZ	12	1.10	0.27				1.86	0.27

Mean gene expression (ratio of measured transcript level to the geometric mean of the measurements for three housekeeping genes) are tabulated for glutamatergic transcripts within each subject group. The large cell population is enriched with glutamatergic relay neurons and the small cells are enriched with GABAergic neurons and astroglia. *Post-hoc* tests were performed for the large cell population, and statistically significant differences ($p<0.05$) in transcript abundance between schizophrenia subjects (SCZ) and the comparison group (NC) are indicated where p values are in bold typeface and underlined. N=sample number, SEM= standard error of mean.