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Abnormalities of the NMDA Receptor and Associated Intracellular Molecules in the Thalamus in Schizophrenia and Bipolar Disorder

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Several lines of investigation support a hypothesis of glutamatergic dysfunction in schizophrenia, including our recent reports of altered NMDA receptor subunit and associated intracellular protein transcripts in the thalamus of elderly patients with schizophrenia. In the present study, we used *in situ* hybridization to measure the expression of NMDA subunits (NR1, NR2A-D), and associated intracellular proteins (NF-L, PSD95, and SAP102) in a second, younger cohort from the Stanley Foundation Neuropathology Consortium, which included patients with both schizophrenia and affective disorders. We wanted to determine whether glutamatergic abnormalities in the thalamus in schizophrenia are present at younger ages, and whether these abnormalities occur in other psychiatric illnesses. In the present work, we observed increased expression of NMDA NR2B subunit transcripts, and decreased expression of all three associated postsynaptic density protein transcripts in schizophrenia. We also found evidence of glutamatergic dysfunction in the thalamus in affective disorders, particularly in bipolar disorder. In particular, we found decreased NF-L, PSD95, and SAP102 transcripts in bipolar disorder, and decreased SAP102 levels in major depression. Interestingly, one of the most consistent findings across diagnostic groups was an abnormality of intracellular signaling molecules that are linked to the NMDA receptor, rather than changes in the receptor subunits themselves. PSD95 and similar scaffolding molecules link the NMDA receptor with intracellular enzymes that mediate signaling, and also provide a physical link between different neurotransmitter systems to coordinate and integrate information from multiple effector systems. Abnormalities of PSD95-like molecules and other intracellular signaling machinery may contribute to dysregulated communication between multiple neurotransmitter systems (such as glutamatergic and dopaminergic systems) that are potentially involved in the neurobiology of schizophrenia and affective disorders.
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INTRODUCTION

The thalamus is composed of numerous topographically organized nuclei that reciprocally project to limbic, sensory, and motor regions of the cerebral cortex. While the thalamus has traditionally been considered a simple relay station, it plays a substantial role in processing and integrating incoming sensory information by not only transmitting information to the cortex but also regulating the ability of the cortex to process this information (Jones, 1998). Numerous post-mortem and *in vivo* imaging studies report structural (Andreasen *et al*, 1994; Buchsbaum *et al*, 1996; Byne *et al*, 2001; Gilbert *et al*, 2001; Pakkenberg, 1990; Popken *et al*, 2000) and functional (Hazlett *et al*, 1999; Silbersweig *et al*, 1995; Tamminga *et al*, 1992) abnormalities of the thalamus in schizophrenia, including reduced thalamic cell number and volume, and decreased metabolism (for a review of these findings see Clinton and Meador-Woodruff, 2003). Despite mounting evidence for structural pathology and thalamic dysfunction in schizophrenia, relatively few studies have examined the neurochemical substrates that may accompany these changes (Clinton *et al*, 2003; Ibrahim *et al*, 2000b; Oke and Adams, 1987; Smith *et al*, 2001a, b).

Thalamocortical projections, corticothalamic projections, and sensory afferents to the dorsal thalamus primarily use glutamate as a neurotransmitter, which activates both ionotropic and metabotropic glutamate receptors expressed throughout the thalamus (Ibrahim *et al*, 2000a; Jones *et al*, 1998). Several lines of investigation implicate glutamatergic dysfunction in schizophrenia (Coyle, 1996; Goff and Wine, 1997; Olney *et al*, 1999). The glutamate hypothesis of schizophrenia is based largely on the observation that NMDA receptor antagonists like phencyclidine can trigger a

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schizophrenia-like syndrome in healthy subjects, and exacerbate symptoms in schizophrenia (Lahti *et al*, 1995). Post-mortem studies have revealed NMDA receptor abnormalities in several limbic structures implicated in schizophrenia, including the prefrontal cortex (Dracheva *et al*, 2001), hippocampus (Gao *et al*, 2000), and thalamus (Ibrahim *et al*, 2000b). Further, clinical trials have shown that treating patients with a combination of standard neuroleptics and drugs that promote NMDA receptor function, such as D-cycloserine, an agonist of the glycine/D-serine co-agonist site of the NMDA receptor, significantly improve negative symptoms (Goff *et al*, 1999, 1995; Goff and Coyle 2001; Javitt *et al*, 1994). Taken together, these data suggest that glutamate, specifically NMDA receptor-mediated, transmission, may be disrupted in schizophrenia.

Glutamate transmission involves myriad molecules, including pre- and postsynaptic receptors and receptor-associated intracellular molecules that link glutamate receptors to signal transduction pathways. The NMDA receptor is a ligand-gated ion channel formed by several subunits, including the obligate NR1 subunit, and combinations of NR2 subunits A–D (Hollmann and Heinemann, 1994). The NR3A and NR3B genes have also been identified, but these subunits are primarily expressed in the brain during development (Das *et al*, 1998), and in the spinal cord (Chatterton *et al*, 2002), respectively. Over the last decade, yeast two-hybrid experiments have identified several glutamate receptor-interacting proteins enriched in the postsynaptic density (PSD), which modulate receptor activity and participate in signal transduction pathways. PSD95, the prototype of this family, contains several domains that bind the C-termini of NMDA NR2 subunits in addition to cytoskeletal elements and signal transduction enzymes. These protein–protein interactions facilitate NMDA receptor function by clustering and anchoring the receptor at the PSD, modulating NMDA receptor sensitivity to glutamate, and, perhaps most importantly, assembling a signaling complex to coordinate NMDA receptor-regulated intracellular processes (Sheng, 2001; Sheng and Pak, 2000). Considering the complexity of the glutamate system, it is possible that glutamate dysfunction in schizophrenia may not involve abnormalities of the NMDA receptor, but instead may be due to a disruption of other molecules involved in glutamate neurotransmission (Clinton *et al*, 2003).

We have recently reported glutamatergic abnormalities in the thalamus of elderly patients with schizophrenia, including decreased expression of NR1 subunit transcripts, decreased binding to the polyamine and glycine sites of the NMDA receptor (Ibrahim *et al*, 2000b), and increased expression of PSD95-like molecules (Clinton *et al*, 2003). These data support a hypothesis of glutamatergic dysfunction in the illness, and indicate that these abnormalities not only involve receptors, but also glutamate receptor-related signal transduction pathways. In the present study, we have repeated these experiments in a second, younger cohort from the Stanley Foundation Neuropathology Consortium, which included patients with both schizophrenia and affective disorders. We wanted to determine whether glutamatergic abnormalities in the thalamus are present at earlier ages in schizophrenia, and whether these abnormalities occur in other psychiatric illnesses.

METHODS

A total of 60 subjects from the Stanley Foundation Neuropathology Consortium were used in these studies. This set consists of 15 patients with schizophrenia, 15 patients with major depressive disorder, 15 patients with bipolar disorder, and 15 nonpsychiatrically ill individuals. A detailed description of this collection has been published (Torrey *et al*, 2000), and a summary of subject characteristics is shown in Table 1. Cryostat sectioned (14 μm) slides were provided to us and stored at −80°C until use. Two slides per subject were prepared for *in situ* hybridization for each probe. Measurements were made after the subject code was broken for analysis of earlier samples received by our lab, so data analysis in this particular study was 'unblinded'.

In situ Hybridization

Riboprobes were synthesized from linearized plasmid DNA containing subclones of NMDA receptor subunits NR1, NR2A-D, and NMDA-associated PSD proteins neurofilament light-chain (NF-L), PSD95, and synapse-associated protein 102 (SAP102), as previously described (Clinton and Meador-Woodruff, 2002; Ibrahim *et al*, 2000b). Briefly, 100 μCi of [^{35}S]UTP (New England Nuclear, Boston, MA) was vacuum dried and 2.0 μl 5 × transcription buffer, 1.0 μl 0.1 DTT, 1.0 μl each of 10 mM ATP, CTP, and GTP, 2.0 μl plasmid DNA, 0.5 μl RNAse inhibitor, and 1.5 μl SP6, T7, or T3 RNA polymerase were added and incubated for 2 h at 37°C. A measusre of 1 μl DNAse (RNAse free) was added to the reaction and incubated for 15 min at room temperature. The labeled probe was purified using a Micro Bio-Spin P-30 Tris Spin Column (Bio-Rad Laboratories).

Table 1 Summary of Subject Characteristics

	Schizophrenia	Bipolar disorder	Major depression	Normal controls
N	15	15	15	15
Age (years)	44.2 (25–62)	42.3 (25–61)	46.4 (30–65)	48.1 (29–68)
Sex	9M, 6F	9M, 6F	9M, 6F	9M, 6F
PMI (h)	33.7 (12–61)	32.5 (13–62)	27.5 (7–47)	23.7 (8–42)
Tissue (pH)	6.1 (5.8–6.6)	6.2 (5.8–6.5)	6.2 (5.6–6.5)	6.3 (5.8–6.6)
Side of brain studied	6R, 9L	8R, 7L	6R, 9L	7R, 8L

Two slides per subject were placed in 4% fomaldehyde at room temperature for 1 h. The slides were then washed in 2 × SSC (300 mM NaCl/30 mM sodium citrate, pH 7.2) three times for 5 min each. Next, the slides were placed in 0.1 M triethanolamine, pH 8.0/acetic anhydride, 400 : 1 (vol : vol), on a stir plate for 10 min. The final wash was in 2 × SSC buffer 5 min, followed by dehydration through graded ethanol washes and air-drying. A coverslip with 500 µl of riboprobe (1 million cpm)/50% formamide buffer/0.01 M DTT was placed on each slide. Slides were placed in a covered tray lined with filter paper saturated with 50% formamide buffer and incubated at 55°C overnight. Approximately 18 h later coverslips were removed and the slides in the NR1, NR2A-D, NF-L, and SAP102 studies were washed in 2 × SSC at room temperature for 10 min, and incubated in RNaseA (200 mg/ml in 10 mM Tris-HCl, pH 8.0/0.5 M NaCl) at 37°C for 30 min. The slides then underwent a series of washes: 2 × SSC for 10 min; 1 × SSC for 10 min at room temperature; 0.5 × SSC at 55°C for 60 min; and 0.5 × SSC for 10 min at room temperature. The slides for the PSD95 studies required different washing conditions. These slides were placed in 2 × SSC at room temperature for 15 min, and then incubated in RNaseA (200 mg/ml in 10 mM Tris-HCl, pH 8.0/0.5 M NaCl) at 37°C for 30 min. Then slides were washed twice in 2 × SSC for 15 min; once in 1 × SSC for 15 min at room temperature; twice in 0.5 × SSC at 55°C for 60 min each; and once in 0.5 × SSC for 15 min at room temperature. Finally, all of the slides were dehydrated in graded ethanol washes, air-dried, and apposed to film (Kodak Biomax MR-1, New England, Nuclear, Boston, MA) for 5–60 days. For each probe, slides from all subjects were processed together to eliminate interassay variability.

Image and Data Analysis

Images were acquired from digitized X-ray films and analyzed using Scion Image Beta 3b for PC. We identified seven discrete thalamic nuclei in each section: anterior (A), central medial (CM), dorsomedial (DM), ventral anterior (VA), ventral lateral (VL), ventral medial (VM), and the reticular nucleus (R). The nuclei were identified based on cellular and white matter patterns defined by cresyl violet staining of sections from each subject, as we have previously described (Clinton *et al*, 2003; Ibrahim *et al*, 2000b). For all experiments, tissue background values from adjacent white matter were subtracted from grayscale values for each nucleus and converted to optical density. The amount of radioactivity bound (in nCi/g) was determined using [^{14}C]microscale standards (Amersham Biosciences, Piscataway, NJ) (Miller, 1991), which were exposed on the same film as the slides for each study. The number of labeled uridine nucleotides contained in each riboprobe and the specific activity of the [^{35}S]UTP were then used to convert bound radioactivity to concentration of mRNA per nucleus, expressed as fmol/g. For all studies, values for each nucleus from two sections per subject were averaged and used for statistical analysis, which was performed for each probe by two-way analysis of variance (ANOVA), with nucleus and diagnosis as independent variables, and mRNA concentrations of each probe as the dependent variable. *Post hoc* analyses were performed using the Neuman–Keuls test. The Kolmogorov–Smirnov test was used to ensure normality of all data. Pearson product moment correlations were used to determine relationships between continuously distributed variables. For all tests $\alpha = 0.05$.

RESULTS

NMDA Subunit Expression in Schizophrenia and Affective Disorders

Transcripts encoding the NMDA subunits NR1, NR2A-D were present in all nuclei studied. They were, however, predominately expressed in the dorsal thalamus, with very low levels present in the reticular nucleus. NR1 transcripts were abundantly expressed throughout the thalamus. NR2B transcripts were also relatively abundant, with moderate levels of NR2A, and low levels of NR2C and NR2D transcripts (Figures 1 and 2). There was a main effect on diagnosis for NR2B mRNA levels ($F = 3.97$, $df = 3, 392$; $p = 0.008$) (Figure 2). *Post hoc* analysis showed that this effect was due to a 30% increase of NR2B transcript expression in the thalamus in schizophrenia. NR2B levels were not significantly altered in either affective disorder compared to controls (Figure 2). There was no main effect on diagnosis for NMDA receptor subunits NR1, NR2A, and 2D, and there were no significant diagnosis × nucleus interactions for any of the NMDA receptor subunits. Correlational analysis showed that NMDA receptor subunit transcript levels did not correlate significantly with age (correlation coefficients ranged from –0.14 to 0.21, p-values = 0.09–0.97). NR2C transcript levels were significantly correlated with post-mortem interval (PMI) ($r = 0.41$, $p = 0.001$). PMI for the schizophrenia group is significantly higher than that of the control group, and our ANOVA for NR2C showed a main effect for NR2C, suggesting that NR2C expression was increased in the thalamus of patients with schizophrenia; however, when we included PMI as a covariate in a subsequent analysis, the effect of diagnosis

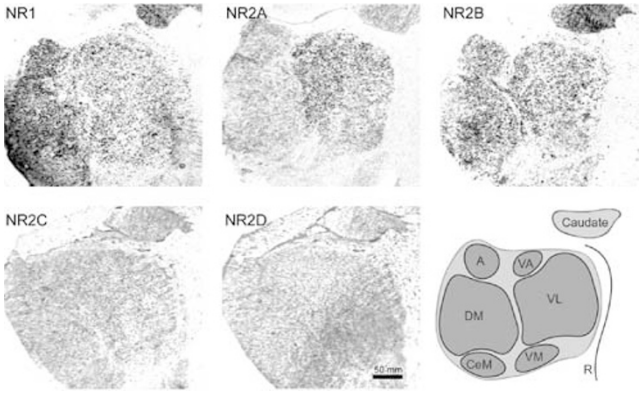

Figure 1 Transcript expression of NMDA receptor subunits NR1, NR2A-D in the human thalamus. NR1 transcripts were heavily expressed across the thalamus. There were also fairly high levels of NR2B transcripts, moderate levels of NR2A, and very low levels of NR2C and NR2D transcripts. In each section, the following nuclei were identified for each subject: anterior (A); central medial (CM); dorsomedial (DM); ventral anterior (VA); ventral lateral (VL); ventral medial (VM), and the reticular nucleus (R). The tail of the caudate nucleus is also readily apparent at this level.

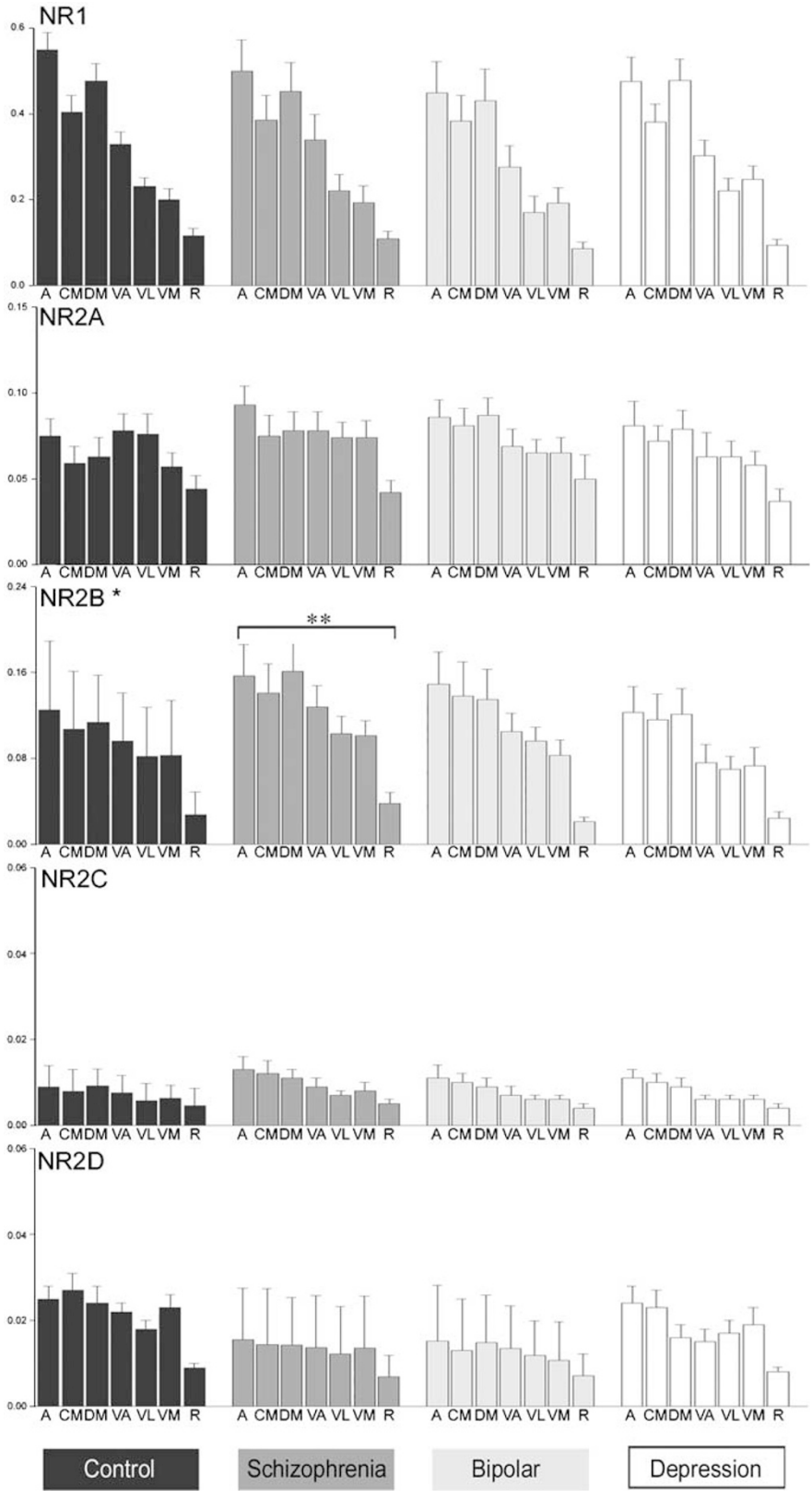

Figure 2 Expression of NMDA receptor subunit transcripts in the thalamus in schizophrenia, bipolar illness, and major depression. NMDA receptor subunit transcripts were measured in seven thalamic nuclei: anterior (A); central medial (CM); dorsomedial (DM); ventral anterior (VA); ventral lateral (VL); ventral medial (VM), and the reticular nucleus (R). Values are mean ± SEM. *Main effect for diagnosis ($p < 0.01$) by two-way ANOVA. ***Post hoc* analysis indicated that NR2B transcript expression was significantly increased in schizophrenia.

on NR2C expression was no longer significant. None of the other NMDA subunit transcripts (NR1, NR2A, NR2B, NR2D) correlated with PMI (correlation coefficients ranged from 0.03 to 0.11, p-values = 0.37–0.75). NR1 transcript levels correlated with pH ($r = 0.54$, $p = 0.000009$), but the other NMDA receptor subunit transcripts did not (correlation coefficients ranged from 0.01 to 0.18, p-values = 0.27–0.84). ANOVA analysis showed that pH levels were not significantly different between diagnostic groups.

NMDA Receptor-Associated PSD Protein Transcripts in Schizophrenia and Affective Disorders

NF-L, PSD95, and SAP102 transcripts were expressed in all nuclei studied. NF-L transcripts were abundantly expressed throughout the thalamus. PSD95 transcripts were also abundant, and SAP102 transcripts were moderately expressed in the thalamus (Figures 3 and 4). There was a main effect for diagnosis on NF-L transcript expression ($F = 7.95$, df = 3,392; $p = 0.00004$). *Post hoc* analysis showed that this effect was due to a 29% decrease of NF-L transcripts in the thalamus of bipolar patients and a 19% decrease in schizophrenia patients, while levels were unchanged in the depression group (Figure 4). There was a main effect for diagnosis on PSD95 mRNA expression ($F = 4.27$, df = 3,392; $p = 0.006$), which was due to a 24 and 19% reduction of transcripts in the thalamus of bipolar and schizophrenia patients, respectively (Figure 4). There was also a main effect for diagnosis on SAP102 transcript expression ($F = 5.08$, df = 3,392; $p = 0.002$). *Post hoc* analysis indicated that this effect was due to 29, 29, and 14% reductions of SAP102 transcripts in bipolar disorder, schizophrenia, and depression, respectively (Figure 4). There were no significant diagnosis × nucleus interactions for any of the NMDA receptor-associated PSD proteins. Correlational analysis showed that PSD protein transcripts did not correlate significantly with either age (correlation coefficients ranged from –0.05 to 0.04, p-values = 0.36–0.69) or PMI (correlation coefficients ranged from –0.19 to –0.07, p-values = 0.13–0.55). All of the PSD protein transcripts did, however, correlate with pH: NF-L ($r = 0.39$, $p = 0.002$), PSD95 ($r = 0.44$, $p = 0.0004$), and SAP102 ($r = 0.41$, $p = 0.001$). In general, pH levels are known to influence mRNA quality and stability, and our results are consistent with this notion since the expression of some transcripts is positively correlated with pH. As mentioned above, ANOVA analysis showed that pH levels were not significantly different between diagnostic groups.

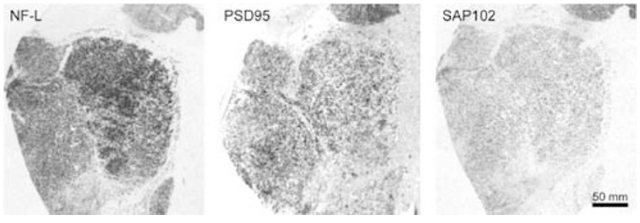

Figure 3 Transcript expression of NMDA receptor-associated PSD proteins NF-L, PSD95, and SAP102 in the human thalamus. NF-L transcripts were abundantly expressed throughout the thalamus. PSD95 transcripts were also heavily expressed in the thalamus, with moderate levels of SAP102 transcripts expression.

DISCUSSION

We have previously reported abnormalities of NMDA receptors and associated intracellular proteins in the thalamus of elderly patients with schizophrenia (Clinton *et al*, 2003; Ibrahim *et al*, 2000b). In the present studies we have again observed changes in the expression of some NMDA receptor subunits and associated PSD proteins in schizophrenia in a younger cohort of patients; however, the pattern of changes differs from our findings in elderly patients. We also found evidence of glutamatergic abnormalities in the thalamus in affective disorders, particularly in bipolar disorder.

Glutamatergic Abnormalities in the Thalamus in Schizophrenia

Several post-mortem studies from our laboratory suggest glutamatergic dysfunction in the thalamus of elderly patients with schizophrenia (Clinton *et al*, 2003; Ibrahim *et al*, 2000b; Smith *et al*, 2001b). We reported reduced expression of NMDA receptor subunit transcripts NR1 and NR2C, and decreased binding at the polyamine and glycine binding sites of the NMDA receptor complex, but did not find prominent changes in the expression of AMPA, kainate, or metabotropic receptors (Ibrahim *et al*, 2000b; Richardson-Burns *et al*, 2000). We subsequently reported a significant increase in the transcript expression of three NMDA receptor-associated PSD proteins, NF-L, PSD95, and SAP102; since these molecules are generally thought to promote NMDA receptor function, the expression of these molecules may be elevated in an attempt to compensate for decreased NMDA receptor expression, or in response to a general deficit in thalamic glutamate neurotransmission (Clinton *et al*, 2003). We have also identified abnormalities of presynaptic vesicular glutamate transporters, which package glutamate for release, and glial-associated excitatory amino transporters that are responsible for removing glutamate from the synaptic cleft, in the thalamus in schizophrenia (Smith *et al*, 2001a, b). Together these data suggest that glutamate neurotransmission is disturbed in the thalamus in schizophrenia, which may be associated with the structural and metabolic thalamic abnormalities previously reported in the illness (Andreasen, 1997; Jones, 1997).

In the present study, we observed a significant increase of NMDA NR2B subunit transcripts, and decreased NF-L, PSD95 and SAP102 transcripts in the thalamus of younger patients with schizophrenia (mean patient age 43 years). These data conflict with our previous work, which reported decreased NMDA NR1 and NR2C transcipts, and *increased* NF-L and SAP102 transcripts in a substantially older group of patients (mean patient age of 70 years) (Clinton *et al*, 2003; Ibrahim *et al*, 2000b). One possible explanation of these contradictory results could be that different stages of the disease are associated with divergent neurochemical changes. The pattern of gene expression for neurotransmitter receptors and associated molecules may vary depending upon the age of a patient, the types of symptoms (ie positive psychotic symptoms, deficit symptoms, or the extent of cognitive impairment) that predominated, or the length of time that the person suffered from the illness. Data

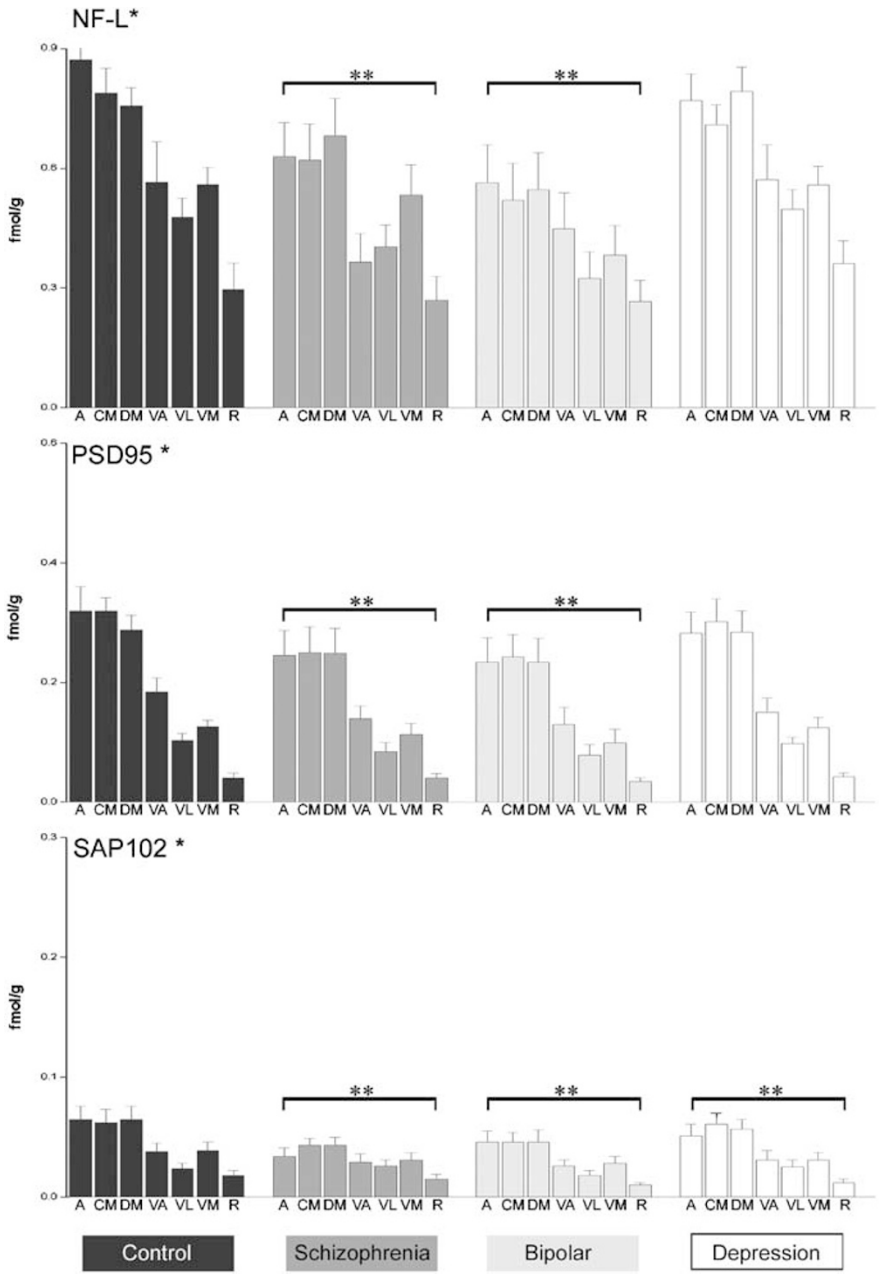

Figure 4 Expression of NMDA receptor subunit transcripts in the thalamus in schizophrenia, bipolar illness, and major depression. NMDA receptor subunit transcripts were measured in seven thalamic nuclei: anterior (A); central medial (CM); dorsomedial (DM); ventral anterior (VA); ventral lateral (VL); ventral medial (VM), and the reticular nucleus (R). Values are mean ± SEM. *Main effect for diagnosis ($p < 0.01$) by two-way ANOVA. ***Post hoc* analysis indicated that NF-L and PSD95 expression was significantly reduced in schizophrenia and bipolar illness. SAP102 transcripts were significantly reduced in all three illnesses.

from these studies suggest that glutamate neurotransmission is altered both at mid-life and later stages of schizophrenia. It remains unclear, though, whether NMDA receptor-related abnormalities represent a primary problem that interferes with normal thalamic function and contributes to symptoms present during mid-course and late stages of schizophrenia, or if these molecular abnormalities occur secondarily to structural or functional thalamic pathology known to exist in the illness.

NMDA receptors are comprised of at least one NR1 subunit, and a combination of NR2 subunits (A–D). The NR2 subunits are differentially expressed in the brain, and each subtype conveys distinct functional properties to the assembled receptor complex. For instance, the inclusion of different NR2 subunits *in vitro* can modulate current amplitudes as well as sensitivity to agonists (like glutamate), co-agonists (such as glycine), and NMDA channel blockers, like Mg^{2+} (Hollmann and Heinemann, 1994). NMDA NR2B subunits are moderately expressed throughout the brain, and in agreement with previous studies, we found moderate levels of NR2B expressed in the thalamus (Ibrahim *et al*, 2000a, b; Jones *et al*, 1998). We also detected a significant increase of NR2B transcripts in the thalamus in schizophrenia, which may be functionally significant. Studies

show that subunit stoichiometry is influenced by neural activity (Audinat *et al*, 1994), so if glutamate transmission is impaired in the thalamus in schizophrenia, it may lead to increased expression and incorporation of NR2B subunits into existing receptors.

Besides subunit composition, NMDA receptor function is also regulated by interactions between the NMDA subunit C-termini and intracellular scaffolding proteins like PSD95, which serve myriad functions, including targeting the receptors to the synaptic membrane, modulating receptor activity, and coupling receptor activation to intracellular signaling pathways. Furthermore, PSD95-like proteins may physically link NMDA receptors to other effector systems in order to integrate information from multiple neurotransmitter receptors (Sheng and Pak, 2000). In the present study, we found reduced expression of PSD95 and SAP102, which are associated with NMDA NR2 subunits. Decreased expression of these proteins may contribute to a breakdown of glutamate receptor-related intracellular signaling. We also observed decreased expression of NF-L, which has been shown to interact with the NMDA NR1 subunit (Ehlers *et al*, 1998) and protein phosphatase-1 (PP1), a major protein/serine/threonine phosphatase that is involved in numerous intracellular processes (Shenolikar, 1994). NF-L may be important for linking NMDA receptors to the synaptic cytoskeleton (Ehlers *et al*, 1998, 1995) and may influence NMDA receptor signaling by anchoring PP1 at the PSD where it can dephosphorylate various PSD proteins, such as NMDA receptor subunits, or CamKII (Terry-Lorenzo *et al*, 2000). Although NF-L may participate in or affect NMDA receptor function, its principle function in the neuron is to interact with the other neurofilament subunits (NF-medium and NF-heavy chain) to maintain the neuronal cytoskeleton (Shaw, 1991). Therefore, while reduced NF-L transcript expression may be related to alterations of glutamate and/or NMDA receptor transmission in the thalamus in schizophrenia, it could also be associated with cytoskeletal changes due to thalamic structural pathology in the illness.

Glutamate Abnormalities and Thalamic Dysfunction in Affective Disorders

The thalamus is a critical link in the corticolimbic circuitry that regulates mood and various cognitive processes, including attention, which can be impaired not only in schizophrenia (Andreasen, 1997), but also in bipolar disorder (Denicoff *et al*, 1999; Sax *et al*, 1999) and major depression (Mialet *et al*, 1996). While there is considerable evidence of thalamic dysfunction in the pathophysiology of schizophrenia, functional or anatomical abnormalities in the thalamus in affective disorders is controversial. A case report in the early 1980s found right thalamic infarction to be associated with secondary mania, suggesting possible thalamic dysfunction in bipolar illness (Bogousslavsky *et al*, 1988; Cummings and Mendez, 1984). More recent *in vivo* imaging studies in patients with affective disorder have rendered mixed results. Some studies reported increased thalamic density (Dewan *et al*, 1988) and volume (Dupont *et al*, 1995; Strakowski *et al*, 1999) in bipolar patients, but other work has found that thalamic volume is either unchanged (Dolan *et al*, 1990; Krishnan *et al*, 1991, 1993; Sax *et al*, 1999; Strakowski *et al*, 1993) or even decreased (Dasari *et al*, 1999; Dupont *et al*, 1995) in bipolar and unipolar depression.

Despite controversial data on possible structural or anatomical abnormalities of the thalamus in bipolar disorder and major depression, there are studies that suggest functional, and possibly neurochemical abnormalities in the thalamus, particularly in bipolar illness. Abnormal blood flow and metabolism have been noted in the medial thalamus in bipolar depression (Buchsbaum *et al*, 1997; Drevets *et al*, 1995), and increased levels of creatine were found in the thalamus, which may be related to altered cellular energy metabolism (Deicken *et al*, 2001). Our data suggest that glutamate neurotransmission may be abnormal in the thalamus of bipolar patients, since we found decreased expression of all three associated PSD proteins examined, NF-L, PSD95, and SAP102. Reduced NF-L transcript expression could be related to thalamic structural pathology in bipolar patients (Soares and Mann, 1997), or may impair NMDA receptor function, as discussed previously. Further, decreased expression of PSD95 and SAP102 in bipolar illness, and decreased SAP102 levels in major depression, may represent a disruption of NMDA receptor-associated signaling and/or the integration of NMDA receptor signals with information from other receptor systems. An earlier study found reduced [3H]cyclic AMP (cAMP) dependent protein kinase binding in cytosolic fractions from the thalamus of bipolar patients, which lends support to the possibility of disrupted intracellular signaling in the thalamus in bipolar illness (Rahman *et al*, 1997).

Limitations of This Study

Several limitations need to be considered in interpreting data from these studies. First, an important limitation of this and all post-mortem studies in psychiatric illness is the possible confounding effect of psychotropic medications, since these drugs potentially regulate the neurochemical systems under study. The majority of patients with schizophrenia and several of the bipolar patients from this study were exposed to neuroleptics at some point in life (Torrey *et al*, 2000). Antipsychotic medications can modulate thalamic metabolism and immediate early gene expression (Cohen and Wan, 1996; Deutch *et al*, 1995; Holcomb *et al*, 1996). They do not, however, appear to affect thalamic NMDA receptor expression (Ulas *et al*, 1993). Currently no studies have directly examined the effect of neuroleptics on the expression of NMDA receptor-associated PSD molecules. However, Dracheva *et al* (2001) reported altered PSD95 and NMDA receptor subunit expression in the prefrontal and occipital corticies in schizophrenia, and found that these transcripts did not differ between patients that were taking antipsychotic medication within 6 weeks of death and those that were medication-free for greater than 6 weeks. Even less is known about the effect of antidepressants and mood stabilizers on the expression of NMDA receptors and associated intracellular molecules. One study showed that antidepressant agents reduce transcript expression for some NMDA receptor subunits in the thalamus, cortex, cerebellum, and striatum of mouse (Boyer *et al*, 1998). While other work indicates that mood stabilizers like lithium can influence NMDA receptor function (Chuang *et al*, 2002; Hashimoto

et al, 2002), no studies to date have examined the effect of these agents on the expression of glutamate receptors.

Conclusions

Several lines of evidence support a hypothesis of glutamatergic dysfunction in schizophrenia, including our recent reports of altered NMDA receptor subunit and PSD protein transcript expression in the thalamus in schizophrenia (Clinton *et al*, 2003; Ibrahim *et al*, 2000b). In the present experiments using a younger patient cohort, although the pattern of changes differs, we have again found evidence of perturbed glutamate neurotransmission in the thalamus in schizophrenia. Further, our data suggest that thalamic glutamate abnormalities may also occur in bipolar disorder. Interestingly, one of the most consistent findings across diagnostic groups was an abnormality of intracellular signaling molecules that are linked to the NMDA receptor, rather than overt changes in the receptor subunits themselves. PSD95 and similar proteins link the NMDA receptor to intracellular enzymes that mediate signaling. Moreover, these molecules may also provide a physical link between different neurotransmitter systems to coordinate and integrate information from multiple effector systems (Sheng and Pak, 2000). Abnormalities of PSD95-like molecules and other intracellular signaling machinery may contribute to dysregulated communication between multiple neurotransmitter systems (such as glutamatergic and dopaminergic systems) that are potentially involved in the neurobiology of schizophrenia and affective disorders.

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