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Lamina-Specific Abnormalities of NMDA Receptor-Associated Postsynaptic Protein Transcripts in the Prefrontal Cortex in Schizophrenia and Bipolar Disorder

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The hypothesis of *N*-methyl-D-aspartate (NMDA) receptor hypofunction in schizophrenia was initially based on observations that blockade of the NMDA subtype of glutamate receptor by noncompetitive antagonists, such as phencyclidine and ketamine, can lead to clinical symptoms similar to those present in schizophrenia. Recently, glutamate has also been implicated in the pathophysiology of the mood disorders. As impaired NMDA receptor activity may be the result of a primary defect in the NMDA receptors themselves, or secondary to dysfunction in the protein complexes that mediate their signaling, we measured expression of both NMDA subunits and associated postsynaptic density (PSD) proteins (PSD95, neurofilament-light (NF-L), and SAP102) transcripts in the dorsolateral prefrontal cortex in subjects with schizophrenia, bipolar disorder, major depression, and a comparison group using tissue from the Stanley Foundation Neuropathology Consortium. We found decreased NR1 expression in all three illnesses, decreased NR2A in schizophrenia and major depression, and decreased NR2C in schizophrenia. We found no changes of NR2B or NR2D. Receptor autoradiography revealed no alterations in receptor binding in any of the illnesses, indicating no change in total receptor number, but taken with the subunit data suggests abnormal receptor stoichiometry. In the same subjects, PSD95 was unchanged in all three illnesses, while reduced NF-L expression was found in schizophrenia, especially in large cells of layer V. SAP102 expression was reduced in bipolar disorder restricted to small cells of layer II and large cells of layer III in bipolar disorder. These alterations likely reflect altered signaling cascades associated with glutamate-mediated neurotransmission within specific cortical circuits in these psychiatric illnesses.
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INTRODUCTION

The hypothesis of *N*-methyl-D-aspartate (NMDA) receptor hypofunction in schizophrenia was initially based on observations that blockade of the NMDA subtype of glutamate receptor by noncompetitive antagonists, such as phencyclidine and ketamine, can lead to clinical symptoms similar to those present in schizophrenia (Hertzmann *et al*, 1990). Subsequent studies have demonstrated that NMDA receptor antagonists can also lead to alterations in physiologic measures, including impaired prepulse inhibition (Duncan *et al*, 2004, 2006; Shoemaker *et al*, 2005; Moy *et al*, 2006) and increased subcortical dopamine release, which are observed in schizophrenia as well (Geyer and Braff, 1987; Grillon *et al*, 1992; McDowd *et al*, 1993; Braff *et al*, 1999).

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Given these observations, the possibility of NMDA hypofunction in schizophrenia is an attractive and viable hypothesis. However, impaired NMDA receptor activity may be the result of a primary defect in the NMDA receptors themselves, or secondary to dysfunction in the postsynaptic proteins that mediate their signaling, leading to diminished NMDA receptor activity. Past studies on the expression of ionotropic glutamate receptors in psychiatric illnesses have focused almost exclusively on schizophrenia, and have concentrated on cortical and medial temporal lobe structures, generally revealing complex region- and receptor-specific abnormalities (McCullumsmith *et al*, 2004). Although previous reports have found alterations of glutamate receptor expression in schizophrenia, it is not well established if these receptors are also abnormal in the mood disorders, even though glutamate transmission abnormalities have been suggested for major depression (MDD) and bipolar disorder (BD) (Belsham, 2001a, b; Meador-Woodruff *et al*, 2001b; McCullumsmith and Meador-Woodruff, 2002; Molnar *et al*, 2003a; Du *et al*, 2004; Mueller and Meador-Woodruff, 2004; Woo *et al*, 2004).

NMDA glutamate receptors are combinations of six subunits, NR1, NR2A–D, and NR3, which form specific

binding sites that provide substrates for the pharmacological regulation of the NMDA receptor. There is a primary binding site for glutamate, and separate polyamine and glycine (D-serine)-binding sites. Glutamate receptors and downstream signaling enzymes are targeted to a postsynaptic specialization of excitatory synapses known as the postsynaptic density (PSD). Apart from subunit composition of receptors, membrane insertion, functionality, and response to glutamate can be regulated by interactions with PSD proteins that can alter receptor sensitivity to glutamate and modulate signaling cascades by linking glutamate receptors to intracellular effector molecules. Proteins containing PDZ motifs play central roles in scaffolding NMDA receptors and linking them to signaling elements (Kennedy, 1997; Craven and Bredt, 1998; Hsueh and Sheng, 1998; Garner *et al*, 2000). The prototypical PDZ protein, PSD95, is a membrane-associated guanylate kinase (MAGUK) that contains three PDZ domains and associates with NMDA receptors. Two other MAGUKs, PSD-93 and synapse-associated protein SAP102, are also expressed in neurons throughout the brain. Some related proteins, such as neurofilament-light (NF-L), link NMDA receptors to the dendritic spine cytoskeleton.

In this study, we hypothesized that there are abnormalities of proteins associated with NMDA-mediated glutamate transmission in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia. Accordingly, we assessed the expression of the NMDA subunits and three of the proteins implicated in their trafficking and signaling (PSD95, SAP102, and NF-L) by *in situ* hybridization, as well as measuring receptor binding with MK801, ifenprodil, CGP39653, and MDL105519 in schizophrenia, MDD, BD, and a comparison group. We predicted that any changes in NMDA receptor-related molecules in schizophrenia may be localized to specific cellular subpopulations in DLPFC, consistent with previous studies that found lamina- and cell-specific somatodendritic alterations in this illness that have been interpreted to suggest decreased synaptic connectivity of those cells. To determine the laminar and cellular specificity of any changes of the PSD proteins in schizophrenia and the mood disorders, cell-level *in situ* hybridization studies were also conducted.

MATERIALS AND METHODS

Subjects and Tissue Preparation

This study was done on post-mortem brains from 60 subjects obtained from the Stanley Foundation Neuropathology Consortium, consisting of 15 subjects each with diagnoses of schizophrenia, BD, MDD, and a comparison group (Torrey *et al*, 2000; Table 1). Serial sections (14 μm thick) were provided by the Stanley Foundation and stored at −80°C.

In Situ Hybridization Histochemistry

Riboprobes were synthesized from linearized plasmids containing subclones of the NMDA receptor subunits (NR1, NR2A–D), and the related intracellular proteins PSD95, SAP102, and NF-L (Table 2). Each NMDA subunit probe recognized all known isoforms and editing variants. Briefly, 10 μl of [^{35}S]-UTP was dried and 2.0 μl 5 × transcription buffer; 1.0 μl 0.1 M dithiothreitol (DTT); 1.0 μl each of 10 mM ATP, CTP, and GTP; 2.0 μl linearized plasmid; 0.5 μl RNase inhibitor; and 1.5 μl T3, T7, or Sp6 RNA polymerase were mixed and incubated for 2 h at 37°C. A total of 1.0 μl DNase (RNase-free) was then added, and the mixture was incubated for 15 min at room temperature. The reaction mixture was separated through spin columns

Table 1 Characterization of Subjects

	Schizophrenia	Major depression	Bipolar disorder	Normal controls
n	15	15	15	15
Age (years)	44.2 (25–62)	46.4 (30–65)	42.3 (25–61)	48.1 (29–68)
Sex (male/female)	9/6	9/6	9/6	9/6
Race	13C, 2A	15C	14C, 1AA	14C, 1AA
PMI (h)	33.7 (12–61)	27.5 (7–47)	32.5 (13–62)	23.7 (8–42)
Tissue pH	6.1 (5.8–6.6)	6.2 (5.6–6.5)	6.2 (5.8–6.5)	6.3 (5.8–6.6)
Side of brain	6 right, 9 left	6 right, 9 left	8 right, 7 left	7 right, 8 left
Suicide	4/15	7/15	9/15	0/15
EtOH use[a]	5/15	3/15	5/15	1/15
Medication[b]	12/15	14/15	12/15	0/15
SSRI	2/15	4/15	2/15	0/15
Lithium	2/15	2/15	4/15	0/15
Antipsychotic	12/15	0/15	6/15	0/15
Other MS[c]	1/15	0/15	8/15	0/15

Abbreviations: C, Caucasian; AA, African-American; A, Asian; EtOH, ethanol; SSRI, selective serotonin reuptake inhibitor; PMI, post-mortem interval; MS, mood stabilizers.
[a]History of active EtOH abuse or dependence at the time of death.
[b]Treatment with psychotropic medication within 6 weeks of the time of death.
[c]Carbamazepine and valproic acid.

Table 2 Probe Characteristics

Gene	Accession number	Number of bases (bp)	Region of gene
NR1	U08263	460	923–1383
NR2A	D13211	668	815–1483
NR2B	NM_012574	808	1–808
NR2C	U08259	768	1–768
NR2D	U08260	783	118–901
PSD95	U83192	396	2113–2603
SAP102	NM_21120	509	437–946
NF-L	NM_006158	420	851–1271

(Micro Bio-Spin P-30 Tris Chromatography Columns, Bio-Rad Laboratories, Richmond, CA) and the purified fraction was eluted. DTT was added to each fraction to a final concentration of 0.01 M.

Two slides per subject for each probe were removed from -80°C storage and placed in 4% (weight:vol) formaldehyde at room temperature for 1 h. They were then rinsed in $2\times$ standard saline citrate (SSC, 300 mM sodium chloride, and 30 mM sodium citrate, pH 7.2) and subsequently treated with 0.1 M triethanolamine (pH 8.0)/acetic anhydride (400 : 1) with stirring for 10 min at room temperature. The sections were rinsed in $2\times$ SSC for 5 min and dehydrated in graded alcohols before air-drying. [^{35}S]-labeled probes were applied diluting $3–5\times10^{6}$ c.p.m. in 400 µl per slide of hybridization buffer (50% deionized formamide, $3\times$ SSC, $1\times$ Denhardt's solution (0.02% polivinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin), 2% yeast tRNA (10 mg/ml), 50 mM sodium phosphate (pH 7.4), and 10% dextran sulfate in sterile water). Slides were covered with glass coverslips, and placed in humid chambers containing an atmosphere saturated with 50% formamide at 55°C overnight.

After 16 h of hybridization, coverslips were removed and sections were washed three times in $2\times$ SSC at room temperature, immersed in RNAse A (200 mg/ml in 10 mM Tris, 0.5 M NaCl, pH 8.0) for 30 min at 37°C, and then washed through descending concentrations of SSC to a final stringency of $0.5\times$ SSC at 55°C for 1 h. Finally, the sections were dehydrated in graded ethanol washes, allowed to air-dry, and placed in X-ray cassettes and exposed to Kodak Biomax MR film (Kodak, Rochester, NY) for 7–14 days.

For each probe, all subjects were run in the same experiment to eliminate interassay variability. Control slides were used to confirm the specificity of each riboprobe, running 'sense-strand'-labeled sections in parallel with those labeled with 'antisense-strand' probes.

Receptor Autoradiography

Receptor autoradiography was carried out under saturating conditions of ligand using assays that have been previously described in detail (Huettner and Bean, 1988; Ransom and Stec, 1988; Sills *et al*, 1991; Hashimoto *et al*, 1994; Baron *et al*, 1996; Siegel *et al*, 1996; White and Vogel, 1996; Healy *et al*, 1998; Healy and Meador-Woodruff, 1999; Ibrahim *et al*, 2000a, b). We examined multiple binding sites on the NMDA receptor, including the intrachannel site (visualized with [^{3}H]MK-801), polyamine site ([^{3}H]ifenprodil), glutamate site ([^{3}H] CGP39653), and glycine coagonist site ([^{3}H]MDL105,519). Slides from these studies were exposed to Amersham (Piscataway, NJ) [^{3}H] Hyperfilm for 2 days ([^{3}H] MDL105,519), 3 days ([^{3}H] ifenprodil), 1 week ([^{3}H] MK-801), or 2 weeks ([^{3}H] CGP39653). Hyperfilm was developed in Kodak D-19 (4 min at 19°C), agitated in 2% acetic acid (30 s), fixed in Kodak Rapid Fix (5 min), washed under running water (10 min), and air-dried.

Image Analysis

Image analysis of in situ *hybridization.* Developed films were digitized and quantitated using NIH Image (v1.56) on a CCD-based imaging system. Each transcript showed a distinct pattern of laminar distribution across the cortical thickness, and gray scale values were obtained from bands with densities different from adjacent lamina (isodense bands). By overlapping images from thionin counterstaining of the same slides, we were able to correlate isodense bands (1–6) to cytoarchitectural cortical layers (I–VI). Isodense bands did not correspond exactly with traditionally defined cortical layers. Gray scale values from isodense bands were corrected for tissue background, and then converted to optical density (OD). OD values were converted to amount of radioactivity bound (nCi/g) determined from calibrated [^{14}C] microscale standards (Miller, 1991). Using the number of uridine residues contained in each molecule, the bound radioactivity value was converted to concentration of mRNA per isodense band, expressed as fmol/g of tissue. The values from two slides per subject were then averaged to obtain a single value for concentration of each mRNA in each isodense band.

Image analysis of receptor autoradiography. Developed films were digitized and quantitated using NIH Image software. For each cortical isodense band, gray scale values from the 'total' binding slides were corrected for nonspecific binding from an adjacent slide, and gray scale value was converted to OD. The final value was averaged from two slides per subject.

Cell-Level Autoradiography

To evaluate the mRNA expression of the PSD proteins at the cellular level, we used high-resolution autoradiographic analysis and silver-grain counting. After exposure to film, we coated hybridized sections with Kodak NTB-2 nuclear emulsion diluted 1 : 1 with sterile water (Kodak). To ensure the consistency of emulsion thickness across sections, we dipped the slides using a Pelco Auto-Dip Emulsion Coater (Ted Pella Inc., Redding, CA), as previously described (Hashimoto *et al*, 2003). The dipping process was at a constant withdrawal speed (64 mm/min) and temperature (43°C). The sections were air-dried for 3 h at room temperature and stored in darkness at 4°C. Test slides were developed at weekly intervals to determine optimal exposure time. After 4–5 weeks, all slides were developed

in Kodak D19 developer for 2 min, washed in distilled water for 30 s, and fixed in Kodak Rapid Fix for 3 min. Following washing in deionized water for 10 min, the sections were counterstained with thionin, dehydrated in graded alcohols, cleared in xylene, and coverslipped in Permount.

Cellular level autoradiography image analysis. For those PSD proteins for which mRNA expression was changed at the lamina level based on film analysis, we determined if alterations were cell-type specific. Using a Microcomputer Imaging Device system and a Leica DM5000 microscope, we counted silver grains/cell from emulsion-dipped slides by sampling four non-overlapping $220 \times 220\,\mu m$ frames arbitrarily selected from each cortical layer. Cells were identified by counterstaining each section with thionin. As treatment with RNase during *in situ* hybridization destroys Nissl bodies, it was difficult to draw the contours of the somata of cells, thus we counted grains from each subject by encircling nuclei of 150–200 labeled cortical cells per lamina of four different diameters, depending on the cortical layer where they were located: large cells of layers II, IV, and VI (18 μm), large cells of layer III (22 μm), large cells of layer V (26 μm), and small cells in all layers (14 μm) (Figure 1). These diameters correspond to those previously described in the human prefrontal cortex (Benes *et al*, 1996). The larger cells include pyramidal neurons in layers II–VI, as well as basket cells (in layers III–VI) and spiny stellate cells (in layer IV), while the smaller cells are a mixture of a number of cell types (bipolar, double bouquet, neuroglia-form, Martinotti, and chandelier cells). Most of those smaller cells are GABAergic, except for bipolar cells that are glutamatergic. In the corresponding dark-field image, the software determined the number of silver grains in each circle. The precision of the obtained measurements was given by the software; for all cases we established the threshold of light intensity and size to be considered as positive labeling (silver grains *vs* dust/artifact), considering positive labeling any number of silver grains above three per cell after subtracting the background. We determined the background value for grain expression for each section by placing circles of the same diameters used for each lamina over underlying white matter, and subtracted that value to get a corrected grains/cell count from each cell. We averaged the corrected number of grains/cell from each circle diameter in each cortical layer from two slides per subject, resulting in two values per cortical layer per subject, one corresponding to large cells (18, 22, or 26 μm) and the other to small cells (14 μm).

Statistical Analysis

The dependent variables in the *in situ* hybridization studies were mRNA concentration for each receptor subunit or PSD protein in each cortical isodense band, for receptor autoradiography was binding in each isodense band, and for cell-level transcript studies was grains/cell. Correlation analysis was carried out to test for associations between post-mortem interval (PMI), age, and pH and each dependent variable. If significant correlations were found, we used analysis of covariance (ANCOVA) to test the effect of diagnosis. When no significant correlations were found, the effect of diagnosis on each dependent variable was determined by factorial analysis of variance (ANOVA), with diagnosis and isodense band treated as the independent variables for film-level studies, and diagnosis, layer, and cell size as independent variables in cell-level transcript studies. *Post hoc* analyses were carried out using Tukey's honest significant difference test. *Post hoc* tests presented in the text contrast individual diagnostic groups with the comparison group. The complete set of comparisons, including between each diagnosis, are presented in Supplementary Table 1, included as Supplementary material. For all tests, $\alpha = 0.05$. We used Statistica (Statsoft, Tulsa, OK) software for Windows for all statistical analyses.

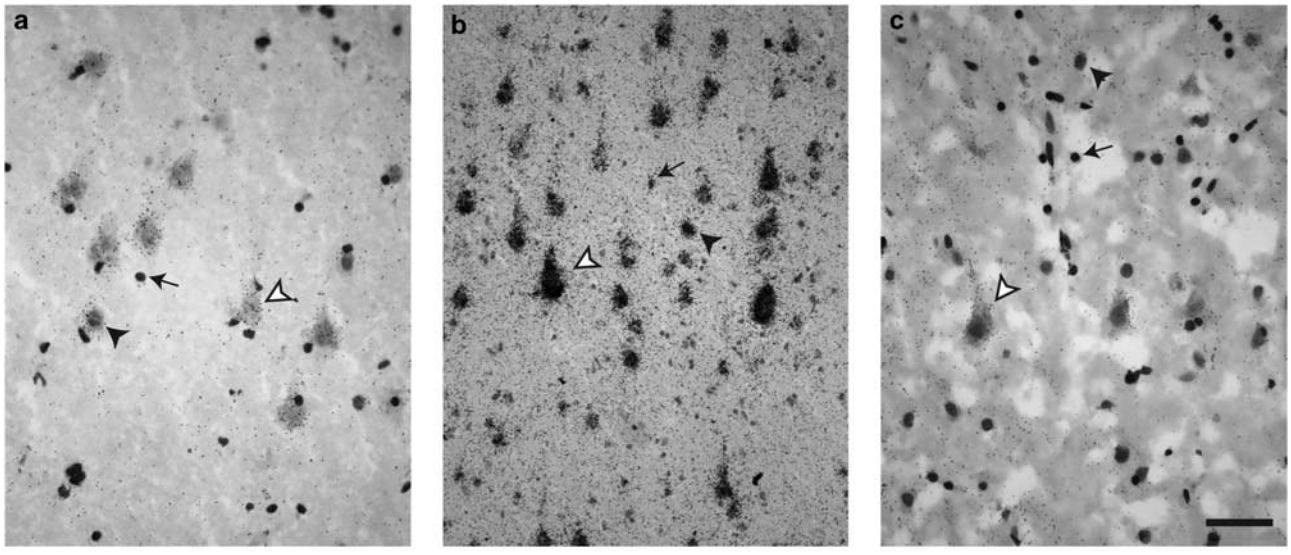


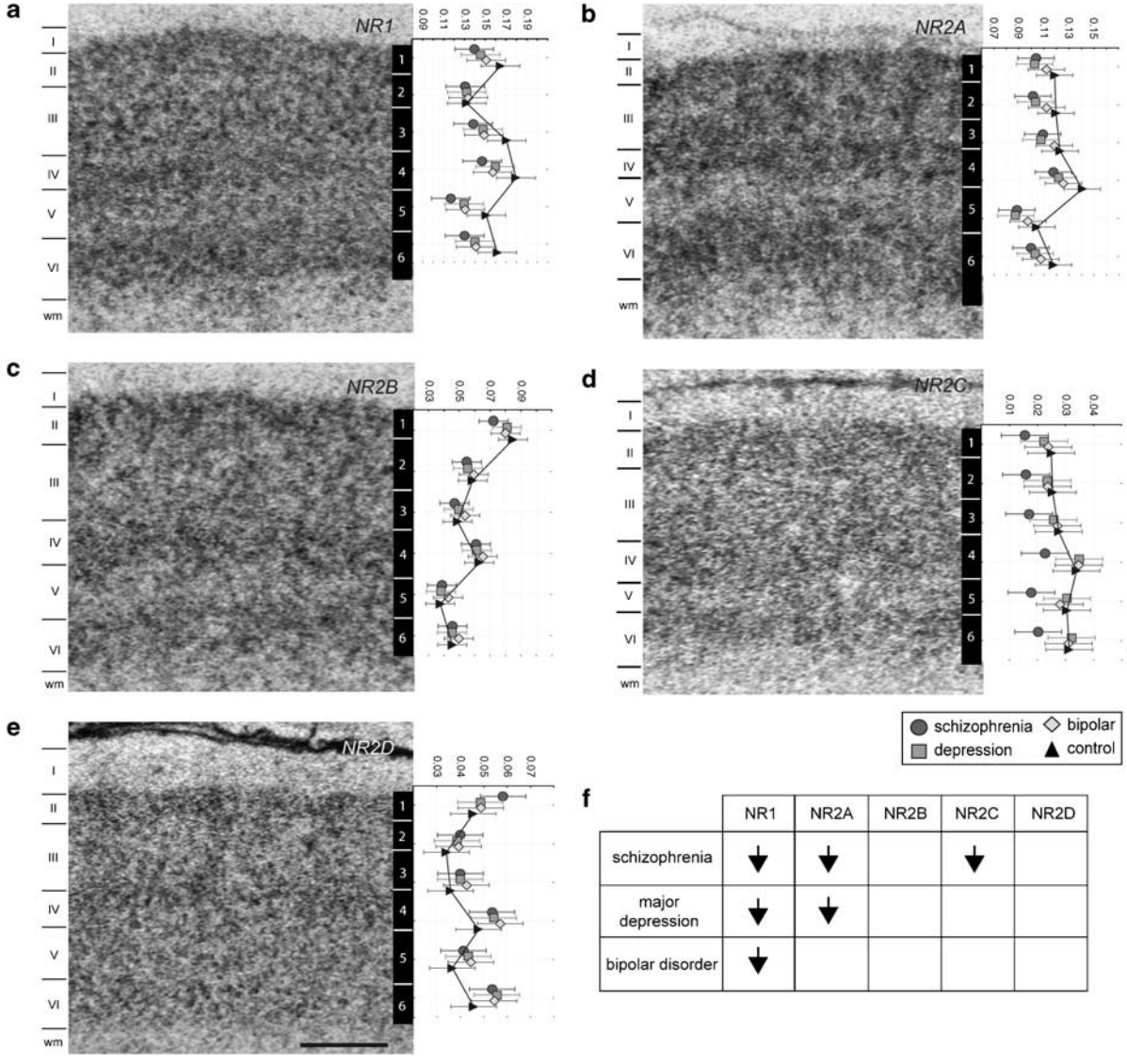
Figure 1 Bright field photomicrographs of cells in cortical layer III hybridized for PSD95 mRNA (a), NF-L (b), and SAP102 (c), showing the distribution of silver grains in large (empty arrowheads) and small cells (black arrowheads), as well as in glial cells (arrows), ×40. Scale bar: 25 μm.

RESULTS

NMDA Receptor Subunits

In situ *hybridization.* NMDA subunit mRNA expression was highest for NR1, followed by NR2A and NR2B; NR2C and NR2D were considerably rarer. All NMDA subunits were expressed in a similar laminar pattern, with three isodense bands of relatively higher intensity corresponding to layers II, IV, and VI (Figure 2).

In situ hybridization experiments revealed an association between pH and the NR1 subunit ($r = 0.41$, $p = 0.0009$) and ANCOVA with pH as covariate revealed a main effect for diagnosis ($F = 3.7$, d.f. $= 3335$, $p = 0.01$) (Figure 2a). *Post hoc* analysis showed a significant decrease of NR1 in schizophrenia ($p < 0.0001$), MDD ($p = 0.004$), and BD ($p = 0.009$) (Figure 2f). NR2A was correlated with pH as well ($r = 0.46$, $p < 0.001$), and ANCOVA revealed a main effect for diagnosis ($F = 3.03$, d.f. $= 3335$, $p = 0.03$) (Figure 2b); *post hoc* tests revealed decreased expression of NR2A in schizophrenia ($p < 0.0001$) and MDD ($p = 0.0002$) (Figure 2f). Regression analysis for NR2B subunit showed significant association with age ($r = 0.3$, $p = 0.02$). ANCOVA indicated a main effect of diagnosis for this subunit ($F = 3.83$, d.f. $= 3335$, $p = 0.01$) that *post hoc* tests revealed as due to difference between groups (BD *vs* schizophrenia, $p = 0.04$) and not compared to control (Figure 2c and f). No associations were found between NR2C expression and pH, PMI or age, and ANOVA revealed a main effect of diagnosis ($F = 8.65$, d.f. $= 3336$, $p < 0.0001$) that *post hoc* testing indicated was due to decreased expression in schizophrenia ($p < 0.0001$) (Figure 2d and f). Regression analysis for NR2D showed no correlation of the expression of this subunit with age, PMI, or pH. ANOVA indicated a main effect for diagnosis ($F = 2.66$, d.f. $= 3336$, $p = 0.05$) but *post hoc* analysis did not reveal any significant difference between groups (Figure 2e and f). We did not



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	NR1	NR2A	NR2B	NR2C	NR2D
schizophrenia	↓	↓		↓	
major depression	↓	↓			
bipolar disorder	↓				

Figure 2 Representative *in situ* hybridization images of transcripts encoding the NMDA receptor subunits in the DLPFC (a–e). The cortical layers for each image are marked in left margin. In the right margin, graphs represent mRNA concentration (fmol/g) in each isodense band for the four subject groups. (f) Table showing significant differences from the comparison group ($p < 0.05$). Scale bar: 1 mm.

detect diagnosis by isodense band interactions for any of the NMDA subunits.

Receptor-binding autoradiography. Specific binding of the four distinct NMDA-binding sites ([^{3}H]MK-801 (intrachannel site), [^{3}H]ifenprodil (polyamine site), [^{3}H]CGP39653 (glutamate site), and [^{3}H]MDL105,519 (glycine site)) was observed in DLPFC (Figure 3). Receptor autoradiography revealed less complex isodense banding patterns than seen from *in situ* hybridization studies of subunit transcripts, with binding sites labeled by NMDA radioligands more homogeneously expressed through cortical layers (Figure 3). [^{3}H]MDL105,519 produced the highest binding density, especially in the infragranular layers (Figure 3h). A slightly different binding pattern was seen for [^{3}H]CGP39653, where a third isodense band corresponding to layers IV–V was noted (Figure 3c). We found no differences by diagnosis in the binding of any of the NMDA ligands (Figure 3).

NMDA Receptor Interacting Proteins

The NMDA-related PSD proteins PSD95, SAP102, and NF-L were highly expressed throughout the DLPFC (Figure 4). Although expressed across all cortical laminae, all three showed the highest level of expression in layers III and V. Grain-counting analyses indicated that all three transcripts are expressed in both large and small cells (Figure 1), likely pyramidal cells and interneurons, respectively. Some glial cells appeared to express all three transcripts as well (Figure 1).

Multiple regression analysis of PSD95 expression revealed an association with both PMI ($r = 0.32$, $p = 0.02$) and pH ($r = 0.41$, $p = 0.002$). ANCOVA with both parameters as covariates showed no main effect of diagnosis for expression of PSD95 transcripts. Cell-level grain-counting experiments confirmed the lack of any changes in PSD95 expression (Figure 4a).

There was an association between the expression of NF-L and pH ($r = 0.35$; $p = 0.01$) but not age or PMI. ANCOVA showed a main effect for diagnosis ($F = 5.35$, d.f. $= 3244$, $p = 0.001$), and *post hoc* analysis revealed decreased expression in schizophrenia ($p < 0.0001$) (Figure 4b). Regression analysis for grains/cell data confirmed the decreased expression of this molecule in schizophrenia compared to controls ($F = 9.03$, d.f. $= 1130$, $p = 0.003$), and was restricted to large cells of layer V ($p = 0.007$) (Figures 1b, 5a and b).

Regression analysis of SAP102 expression showed no associations with pH, PMI, or age. ANOVA for SAP102 revealed a main effect for diagnosis ($F = 4.78$, d.f. $= 3245$, $p = 0.003$) but no diagnosis by isodense band interaction. *Post hoc* analysis revealed a decrease in mRNA expression in BD ($p = 0.08$) compared to controls (Figure 4c). Grain counting in the bipolar group confirmed decreased expression of SAP102 mRNA, but exclusively in the small cells of layer II ($p = 0.02$) and in the large cells of layer III ($p = 0.01$) (Figures 1c, 5c and d).

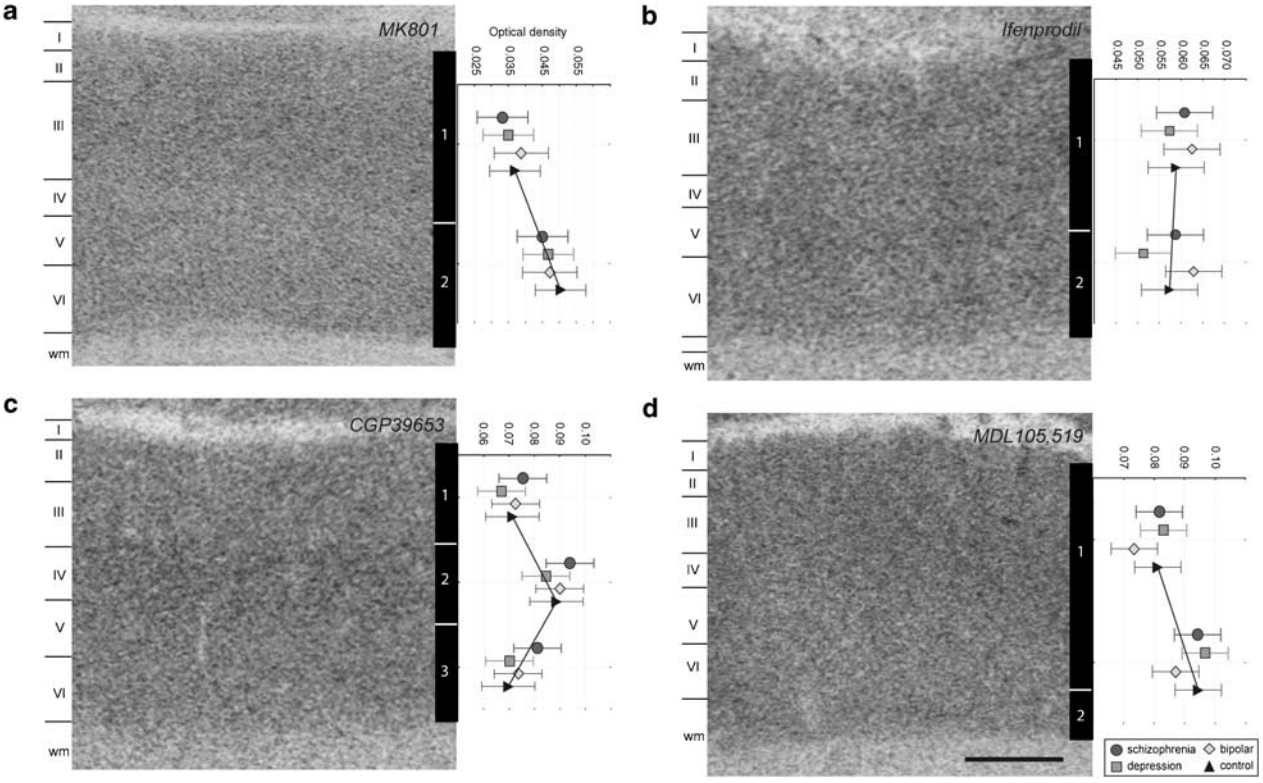

Figure 3 Representative photomicrographs of NMDA receptor-binding autoradiography for (a) [^{3}H]MK801, (b) [^{3}H]ifenprodil, (c) [^{3}H]CGP39653, and (d) [^{3}H]MDL105,519. The cortical layers for each image are marked in left margin. In the right margin, graphs represent ligand binding in each isodense band for the four subject groups. No significant differences from the comparison group were found. Scale bar: 1 mm.

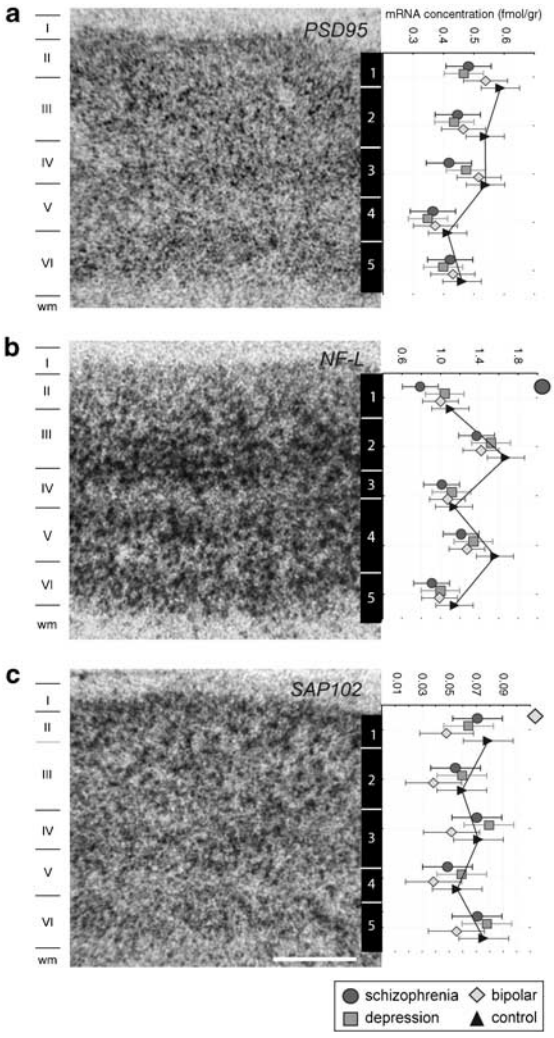

Figure 4 Representative *in situ* hybridization images of transcripts encoding the NMDA-related postsynaptic density (PSD) proteins. (a) PSD95, (b) NEL, and (c) SAP102. The cortical layers for each image are marked in the left margin. In the right margin, graphs represent mRNA concentration (fmol/g) in each isodense band for the four subject groups. *Significant differences from the comparison group ($p<0.05$). SAP102 was decreased in bipolar subjects, and neurofilament-light (NF-L) in schizophrenia. Scale bar: 1 mm.

We carried out correlation analysis of the expression of the NMDA subunits and their related postsynaptic proteins, often finding positive correlations as for NR1 and NR2A ($p<0.0001$) and NR2B ($p<0.05$), and for NR1 and NF-L ($p<0.0001$) in schizophrenia, as expected by the known interaction pattern of the proteins comprising the NMDA receptor complex.

DISCUSSION

We found significant changes in the expression of NMDA receptor subunit transcripts in schizophrenia and mood disorders in DLPFC. Some previous publications found no changes in NMDA receptor expression in these illnesses (Kornhuber *et al*, 1989; Akbarian *et al*, 1996b). However, our results are consistent with other reports that have identified significant changes in ionotropic receptors in schizophrenia (Meador-Woodruff *et al*, 2001a). A shift in the relative proportion of transcripts encoding the NR2 subunits was found in schizophrenia by Akbarian *et al* (1996a), a finding that correlates with our *in situ* hybridization results. Using reverse transcriptase polymerase chain reaction (RT–PCR), decreased NR1 mRNA in frontal cortex was found (Sokolov, 1998), while Dracheva *et al* (2001) found increased expression of NR1 and NR2A but not NR2B transcripts in DLPFC and occipital cortex by RT–PCR. The lack of correspondence between some of our results in schizophrenia and several previous reports of ionotropic receptor expression abnormalities in DLPFC may be due to several factors that could affect mRNA expression. Differences between experimental techniques and subjects characteristics (eg hospitalization status, medication exposure, and age of patients at the time of death) are key factors that may be associated with discrepancies between post-mortem studies.

The abnormal expression of NMDA subunits, together with the lack of changes in receptor binding, suggests a possible change in the stoichiometry of the receptor, which might affect its pharmacology and response to glutamate, as well as its insertion in the postsynaptic membrane. It is interesting that the most robust changes in DLPFC in the three illnesses included in this study involve the 'obligatory' subunit for normal NMDA receptor function, NR1. There is a multitude of cellular mechanisms that regulate the assembly of NMDA receptor subunits into functional channels, endoplasmic reticulum (ER) retention or release, and intracellular trafficking and eventual synaptic delivery (McIlhinney *et al*, 1998, 2003; Standley *et al*, 2000; Meddows *et al*, 2001; Roche *et al*, 2001; Sans *et al*, 2001, 2003; Barria and Malinow, 2002, 2005; Wenthold *et al*, 2003a, b). Demonstration of these effects is found in interesting results from a selective knockout of the NR1 gene, which demonstrated that disrupting NMDA receptor subunit composition impairs normal subcellular targeting of NMDA channels (Fukaya *et al*, 2003). NR1 deletion in the CA1 hippocampal region leads to retention of the NR2B subunit in the lumen of the ER and accumulation of this subunit in the perikarya. This alteration in ER transit results in reduced expression of the NR2B subunit in NMDA receptors in dendrites (Fukaya *et al*, 2003).

Alterations of glutamate subunit expression not only can change the electrophysiology of the receptors themselves, but can also change the probability of coupling the receptor to intracellular proteins that mediate many intracellular processes. Pharmacological and post-mortem studies suggest that alterations of glutamatergic neurotransmission occur in schizophrenia and the mood disorders. While this is often assumed to be due to changes in glutamate receptor expression, these alterations might also be a consequence of abnormal protein interactions that could result in misplacement of glutamate receptors in different cellular compartments, lack of stabilization of the receptors in the PSD, abnormal binding to intracellular effector molecules, or dysregulation of receptor turnover. These processes are mediated by, among others, the PSD proteins in the present study.

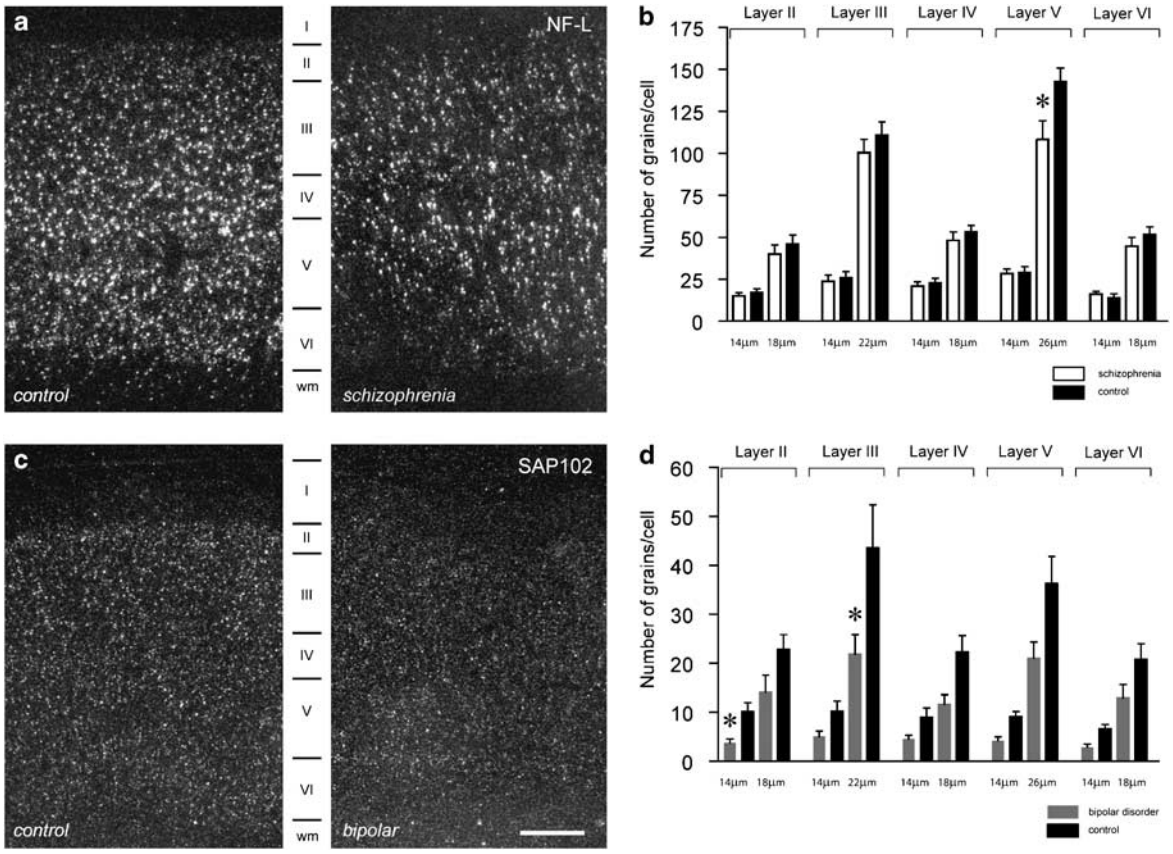

Figure 5 Cellular grain density data for the expression of neurofilament-light (NF-L) in schizophrenia (a and b) and SAP102 in BD (c and d) and the comparison group. Limits between cortical layers are noted between the photomicrographs (dark field, × 2.5). Each graph demonstrates grain/cell in both small cells and large cells assayed in each cortical lamina. Diameters of the circles used in each cortical layer are shown. *Significant differences from the comparison group ($p < 0.05$). Scale bar: 500 μm.

Abnormalities of NMDA-Associated Proteins in Schizophrenia and Mood Disorders

NMDA receptors are anchored in the postsynaptic membrane and linked to a large complex of intracellular proteins, including PSD95, SAP102, and NF-L. While PSD95 and SAP102 bind exclusively to NR2 subunits, NF-L binds to NR1. In our experiments, we found no changes in PSD95 expression, but decreased transcripts encoding NF-L in schizophrenia and SAP102 in BD. Previous studies have examined PSD95 expression in schizophrenia, and although a previous study found decreased transcript expression in DLPFC (Ohnuma *et al*, 2000), our results coincide with those that found no changes in PSD95 expression in the cerebral cortex by RT–PCR (Dracheva *et al*, 2005), immunoautoradiography (Toro and Deakin, 2005), and western blot analysis (Toyooka *et al*, 2002). Toyooka *et al* (2002) found decreased SAP102 protein expression in the hippocampus but not DLPFC in schizophrenia. Abnormal SAP102 expression in BD could affect the regulation of NMDA receptor exocytosis in the PSD, as SAP102 is a key protein in the exocyst complex that transports NMDA receptors from the ER to the postsynaptic membrane (Sans *et al*, 2003).

Previous reports suggest that the intracellular machinery that is coupled to the NMDA receptor subunits is abnormal in BD in the hippocampus (McCullumsmith *et al*, 2007) and in the thalamus (Clinton and Meador-Woodruff, 2004). Consistent with our results, both studies found decreased expression of transcripts for SAP102 in BD. These data support our results, suggesting that BD might be associated with abnormalities of glutamate-linked intracellular signaling and trafficking processes.

While PSD95 and SAP102 bind to NR2 subunits, NF-L binds to NR1 and maintains the stability of the NMDA receptor in the PSD by binding actin filaments of the cytoskeleton of the dendritic spine (Ehlers *et al*, 1995, 1998). This characteristic suggests that decreased NF-L expression in schizophrenia might be part of a more generalized cytoskeletal disruption in the cell as a response to the illness, or alternatively be associated with the decreased expression of this subunit in the same subjects. This suggests a possible instability of NMDA receptors in the postsynaptic membrane that may affect recycling and turnover at the spine, perhaps altering the number of receptors available at the membrane or their clustering at the synapse. Apart from trafficking and stabilizing NMDA receptors at the PSD, NF-L also interacts with protein phosphatase-1 (PP1), an important protein/serine/threonine phosphatase that modulates numerous intracellular pathways (Shenolikar, 1995; Terry-Lorenzo *et al*, 2000). It has been proposed that NF-L might bind PP1 and position it

to dephosphorylate other proteins such as CaMKII, actin, or NMDA receptors (Shenolikar, 1995; Terry-Lorenzo *et al*, 2000), mechanisms that could also be affected by the reduction of NF-L expression we found in schizophrenia. Future examination of posttranslational processes will need to be performed to confirm this hypothesis.

Cellular and Laminar Abnormalities in PSD Proteins Expression

Our data from cell-level mRNA expression analysis reveal that alterations in NF-L expression in DLPFC in schizophrenia are exclusively located in the large cells of cortical layer V. Histograms of labeled *vs* nonlabeled cells showed no subpopulation of cells expressing PSD proteins of each cell size (large *vs* small cells) (data not shown), patterns shown for some other proteins in the prefrontal cortex (Hashimoto *et al*, 2005) and striatum (Gerfen *et al*, 1991). This suggests that the vast majority of cells in which we saw differences in PSD protein expression are pyramidal cells of layers III and V. Thalamocortical input to the pyramidal cells of the DLPFC almost exclusively innervate layer IV, but those terminals have been shown to make synaptic contact with the apical dendrites of the pyramidal cells of layer V (Staiger *et al*, 2004). Interestingly, layer V receive also modest projections from the spiny stellate cells of layer IV, where most thalamocortical afferents are located. Recent studies have shown that layer-IV spiny neurons form a highly interconnected local excitatory network that is able to amplify weak thalamic input before it is relayed to the supragranular layers (Stratford *et al*, 1996; Feldmeyer *et al*, 1999, 2002). Our results suggest a dysregulation of the expression of the molecules implicated in glutamatergic signaling in layer V in schizophrenia. This may produce abnormal reception of thalamocortical and layer-III and -IV synapses, affecting afferent cortical and subcortical circuits.

In BD we detected decreased expression of SAP102, exclusively in small cells of layer II and large cells of layer III of the DLPFC. Abnormal neurotransmission in projecting layer-III cells would have an effect on their ipsi- and contralateral projections to layers II and III, and locally to the apical dendrites of the pyramidal cells of layer V, while dysfunction of small layer-II cells, probably interneurons, would result in abnormalities in signal transmission modulation of inputs from layer III to layer II pyramidal cells and their subsequent corticocortical connections to other areas.

Our finding of expression of NMDA receptor-related intracellular proteins in glial cells require special mention. Aside from their important role in removing glutamate from the extracellular medium, astrocytes have been shown to respond to glutamate via activation of specific receptors. Using pharmacological and molecular approaches, astroglial expression of AMPA and kainate receptors is established (Burnashev *et al*, 1992; Condorelli *et al*, 1993, 1999; Jabs *et al*, 1994; Seifert and Steinhauser, 1995; Seifert *et al*, 1997; Verkhratsky and Steinhauser, 2000; Burnashev, 2005) and functional NMDA receptor expression in astrocytes has been demonstrated recently. Analysis of the functional expression of NMDA receptors in astrocytes revealed significant differences from neuronal NMDA receptors (Schipke *et al*, 2001). Subunits of NMDA receptors have been detected in astrocytes by immunohistochemistry, *in situ* hybridization, and PCR (Schipke *et al*, 2001). Several functional studies have demonstrated NMDA-mediated currents and NMDA-induced $[Ca^{2+}]$ responses in astrocytes (Shao and McCarthy, 1997; Ziak *et al*, 1998; Kondoh *et al*, 2001; Schipke *et al*, 2001). Recordings from astrocytes in slices revealed slow currents (Schipke *et al*, 2001), which did not resemble classic NMDA receptor-mediated responses (Lalo *et al*, 2006; Verkhratsky and Kirchhoff, 2007). In a very elegant study, Lalo *et al* (2006) identified NMDA-induced currents in cortical astrocytes, and showed that these currents can also be activated by glutamate released after stimulation of neuronal synaptic terminals as well as during spontaneous release of neurotransmitter. Our results showing expression of NMDA receptor-associated intracellular proteins further support the existence of functional NMDA receptors in cortical astrocytes.

LIMITATIONS OF THE STUDY

The direct study of the post-mortem human brain is critical in the study of psychiatric disorders, but also has important limitations. For example, markers of the quality of mRNA need to be considered. In our case, we often found significant correlations between PMI, brain pH, or age of the subjects, and the expression of the molecules studied. It has been shown that the impact of PMI on mRNA integrity in post-mortem studies is secondary to agonal status and freezing of the tissue (Harrison *et al*, 1995, 1991; Kingsbury *et al*, 1995; Yates *et al*, 1990). In this study, PMI was correlated with the expression of several of the molecules studied, and in those cases we used PMI as a covariate in the data analysis.

Although enzyme-activity measures can be affected by brain pH (Yates *et al*, 1990; Taylor *et al*, 1986; Perry *et al*, 1982), immunoreactivity of brain proteins and ligand binding to receptors do not appear to vary substantially as a function of brain pH (Harrison *et al*, 1995). However, tissue levels of many mRNAs can be related to brain pH (Harrison *et al*, 1995; Kingsbury *et al*, 1995). Based on observations that sudden deaths are associated with brain pH of $\sim$6.8 and prolonged agonal states may produce a brain pH of $<$6.0, Harrison and Kleinman (2000) have recommended that all brains be screened for pH and those with values $<$6.1 be excluded from study, a recommendation that we followed in this study.

Age has been shown to be an important factor in glutamate receptor binding and mRNA expression in rodents (Magnusson, 1998, 2000; Nicolle and Baxter, 2003). We found no significant correlations with age and NMDA binding of any of the ligands we used in this study, and except for NR2B, no associations with NMDA subunit expression and age.

In conclusion, our data suggest that both schizophrenia and BD are characterized by alterations in the transcript expression of subunits of the NMDA receptor, as well as associated intracellular proteins in the DLPFC in both illnesses. The identification of the abnormal expression of these molecules related to glutamate receptor trafficking and signaling provides evidence for the involvement of the

glutamate system in the pathophysiology of schizophrenia and mood disorders.

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DISCLOSURE/CONFLICT OF INTEREST

JH Meador-Woodruff is the editor-in-chief of this journal and receives an honorarium from ACNP; he has no other conflicts of interest. Dr Beneyto has no conflicts of interests.

REFERENCES

Akbarian S, Sucher NJ, Bradley D, Tafazzoli A, Trinh D, Hetrick WP *et al* (1996a). Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. *J Neurosci* **16**: 19–30.

Akbarian S, Sucher NJ, Bradley D, Tafazzoli A, Trinh D, Hetrick WP *et al* (1996b). Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. *J Neurosci* **16**: 19–30.

Baron BM, Siegel BW, Harrison BL, Gross RS, Hawes C, Towers P (1996). [3H]MDL 105,519, a high-affinity radioligand for the *N*-methyl-D-aspartate receptor-associated glycine recognition site. *J Pharmacol Exp Ther* **279**: 62–68.

Barria A, Malinow R (2002). Subunit-specific NMDA receptor trafficking to synapses. *Neuron* **35**: 345–353.

Barria A, Malinow R (2005). NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. *Neuron* **48**: 289–301.

Belsham B (2001a). Glutamate and its role in psychiatric illness. *Hum Psychopharmacol* **16**: 139–146.

Belsham B (2001b). Glutamate and its role in psychiatric illness. *Hum Psychopharmacol* **16**: 139–146.

Benes FM, Vincent SL, Marie A, Khan Y (1996). Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. *Neuroscience* **75**: 1021–1031.

Braff DL, Swerdlow NR, Geyer MA (1999). Symptom correlates of prepulse inhibition deficits in male schizophrenic patients. *Am J Psychiatry* **156**: 596–602.

Burnashev N (2005). Dynamic modulation of AMPA receptor-mediated synaptic transmission by polyamines in principal neurons. Focus on 'polyamines modulate AMPA receptor-dependent synaptic response in immature layer *v* pyramidal neurons'. *J Neurophysiol* **93**: 2371.

Burnashev N, Khodorova A, Jonas P, Helm PJ, Wisden W, Monyer H. *et al* (1992). Calcium-permeable AMPA-kainate receptors in fusiform cerebellar glial cells. *Science* **256**: 1566–1570.

Clinton SM, Meador-Woodruff JH (2004). Abnormalities of the NMDA receptor and associated intracellular molecules in the thalamus in schizophrenia and bipolar disorder. *Neuropsychopharmacology* **29**: 1353–1362.

Condorelli DF, Conti F, Gallo V, Kirchhoff F, Seifert G, Steinhauser C *et al* (1999). Expression and functional analysis of glutamate receptors in glial cells. *Adv Exp Med Biol* **468**: 49–67.

Condorelli DF, Dell'Albani P, Corsaro M, Barresi V, Giuffrida Stella AM (1993). AMPA-selective glutamate receptor subunits in astroglial cultures. *J Neurosci Res* **36**: 344–356.

Craven SE, Bredt DS (1998). PDZ proteins organize synaptic signaling pathways. *Cell* **93**: 495–498.

Dracheva S, Marras SA, Elhakem SL, Kramer FR, Davis KL, Haroutunian V (2001). *N*-methyl-D-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with schizophrenia. *Am J Psychiatry* **158**: 1400–1410.

Dracheva S, McGurk SR, Haroutunian V (2005). mRNA expression of AMPA receptors and AMPA receptor binding proteins in the cerebral cortex of elderly schizophrenics. *J Neurosci Res* **79**: 868–878.

Du J, Gray NA, Falke CA, Chen W, Yuan P, Szabo ST *et al* (2004). Modulation of synaptic plasticity by antimanic agents: the role of AMPA glutamate receptor subunit 1 synaptic expression. *J Neurosci* **24**: 6578–6589.

Duncan GE, Moy SS, Lieberman JA, Koller BH (2006). Effects of haloperidol, clozapine, and quetiapine on sensorimotor gating in a genetic model of reduced NMDA receptor function. *Psychopharmacology (Berl)* **184**: 190–200.

Duncan GE, Moy SS, Perez A, Eddy DM, Zinzow WM, Lieberman JA *et al* (2004). Deficits in sensorimotor gating and tests of social behavior in a genetic model of reduced NMDA receptor function. *Behav Brain Res* **153**: 507–519.

Ehlers M, Fung E, O'Brien R, Huganir R (1998). Splice variant-specific interaction of the NMDA receptor subunit NR1 with neuronal intermediate filaments. *J Neurosci* **18**: 720–730.

Ehlers MD, Tingley WG, Huganir RL (1995). Regulated subcellular distribution of the NR1 subunit of the NMDA receptor. *Science* **269**: 1734–1737.

Feldmeyer D, Egger V, Lubke J, Sakmann B (1999). Reliable synaptic connections between pairs of excitatory layer 4 neurones within a single 'barrel' of developing rat somatosensory cortex. *J Physiol* **521**(Part 1): 169–190.

Feldmeyer D, Lubke J, Silver RA, Sakmann B (2002). Synaptic connections between layer 4 spiny neurone-layer 2/3 pyramidal cell pairs in juvenile rat barrel cortex: physiology and anatomy of interlaminar signalling within a cortical column. *J Physiol* **538**: 803–822.

Fukaya M, Kato A, Lovett C, Tonegawa S, Watanabe M (2003). Retention of NMDA receptor NR2 subunits in the lumen of endoplasmic reticulum in targeted NR1 knockout mice. *Proc Natl Acad Sci USA* **100**: 4855–4860.

Garner CC, Nash J, Huganir RL (2000). PDZ domains in synapse assembly and signalling. *Trends Cell Biol* **10**: 274–280.

Gerfen CR, McGinty JF, Young III WS (1991). Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: *in situ* hybridization histochemical analysis. *J Neurosci* **11**: 1016–1031.

Geyer MA, Braff DL (1987). Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophr Bull* **13**: 643–668.

Grillon C, Ameli R, Charney DS, Krystal J, Braff D (1992). Startle gating deficits occur across prepulse intensities in schizophrenic patients. *Biol Psychiatry* **32**: 939–943.

Harrison PJ, Heath PR, Eastwood SL, Burnet PW, McDonald B, Pearson RC (1995). The relative importance of premortem acidosis and postmortem interval for human brain gene expression studies: selective mRNA vulnerability and comparison with their encoded proteins. *Neurosci Lett* **200**: 151–154.

Harrison PJ, Kleinman JE (2000). Methodological issues. In: Harrison PJ, Roberts GW (eds). *The Neuropathology of Schizophrenia*. Oxford University Press: New York, pp 339–350.

Harrison PJ, Procter AW, Barton AJ, Lowe SL, Najlerahim A, Bertolucci PH *et al* (1991). Terminal coma affects messenger RNA detection in post mortem human temporal cortex. *Brain Res Mol Brain Res* **9**: 161–164.

Hashimoto K, Mantione CR, Spada MR, Neumeyer JL, London ED (1994). Further characterization of [3H]ifenprodil binding in rat brain. *Eur J Pharmacol* **266**: 67–77.

Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z *et al* (2003). Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci* **23**: 6315–6326.

Hashimoto T, Arion G, Unger T, Volk DW, Mirnics K, Lewis DA (2005). Analysis of gaba-specific transcriptome in the prefrontal cortex of subjects with schizophrenia. *Soc Neurosci* Abstr 35, 675.4.

Healy DJ, Haroutunian V, Powchik P, Davidson M, Davis KL, Watson SJ *et al* (1998). AMPA receptor binding and subunit mRNA expression in prefrontal cortex and striatum of elderly schizophrenics. *Neuropsychopharmacology* **19**: 278–286.

Healy DJ, Meador-Woodruff JH (1999). Ionotropic glutamate receptor modulation of 5-HT6 and 5-HT7 mRNA expression in rat brain. *Neuropsychopharmacology* **21**: 341–351.

Hertzmann M, Reba RC, Kotlyarov EV (1990). Single photon emission computed tomography in phencyclidine and related drug abuse. *Am J Psychiatry* **147**: 255–256.

Hsueh YP, Sheng M (1998). Anchoring of glutamate receptors at the synapse. *Prog Brain Res* **116**: 123–131.

Huettner JE, Bean BP (1988). Block of *N*-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proc Natl Acad Sci USA* **85**: 1307–1311.

Ibrahim H, Healy D, Hogg AJ, Meador-Woodruff J (2000a). Nucleus-specific expression of ionotropic glutamate receptor subunit mRNAs and binding sites in primate thalamus. *Brain Res Mol Brain Res* **79**: 1–17.

Ibrahim HM, Hogg Jr AJ, Healy DJ, Haroutunian V, Davis KL, Meador-Woodruff JH (2000b). Ionotropic glutamate receptor binding and subunit mRNA expression in thalamic nuclei in schizophrenia. *Am J Psychiatry* **157**: 1811–1823.

Jabs R, Kirchhoff F, Kettenmann H, Steinhauser C (1994). Kainate activates Ca(2+)-permeable glutamate receptors and blocks voltage-gated K+ currents in glial cells of mouse hippocampal slices. *Pflugers Arch* **426**: 310–319.

Kennedy MB (1997). The postsynaptic density at glutamatergic synapses. *Trends Neurosci* **20**: 264–268.

Kingsbury AE, Foster OJ, Nisbet AP, Cairns N, Bray L, Eve DJ *et al* (1995). Tissue pH as an indicator of mRNA preservation in human post-mortem brain. *Brain Res Mol Brain Res* **28**: 311–318.

Kondoh T, Nishizaki T, Aihara H, Tamaki N (2001). NMDA-responsible, APV-insensitive receptor in cultured human astrocytes. *Life Sci* **68**: 1761–1767.

Kornhuber J, Mack-Burkhardt F, Riederer P, Hebenstreit GF, Reynolds GP, Andrews HB *et al* (1989). [3H]MK-801 binding sites in postmortem brain regions of schizophrenic patients. *J Neural Transm* **77**: 231–236.

Lalo U, Pankratov Y, Kirchhoff F, North RA, Verkhratsky A (2006). NMDA receptors mediate neuron-to-glia signaling in mouse cortical astrocytes. *J Neurosci* **26**: 2673–2683.

Magnusson KR (1998). Aging of glutamate receptors: correlations between binding and spatial memory performance in mice. *Mech Ageing Dev* **104**: 227–248.

Magnusson KR (2000). Declines in mRNA expression of different subunits may account for differential effects of aging on agonist and antagonist binding to the NMDA receptor. *J Neurosci* **20**: 1666–1674.

McCullumsmith RE, Clinton SM, Meador-Woodruff JH (2004). Schizophrenia as a disorder of neuroplasticity. *Int Rev Neurobiol* **59**: 19–45.

McCullumsmith RE, Meador-Woodruff JH (2002). Striatal excitatory amino acid transporter transcript expression in schizophrenia, bipolar disorder, and major depressive disorder. *Neuropsychopharmacology* **26**: 368–375.

McCullumsmith RE, Kristiansen LV, Beneyto M, Scarr E, Dean B, Meador-Woodruff JH (2007). Decreased NRI, NR2A, and SAP102 transcript expression in the hippocampus in bipolar disorder. *Brain Res* **1127**: 108–118.

McDowd JM, Filion DL, Harris MJ, Braff DL (1993). Sensory gating and inhibitory function in late-life schizophrenia. *Schizophr Bull* **19**: 733–746.

McIlhinney RA, Le Bourdelles B, Molnar E, Tricaud N, Streit P, Whiting PJ (1998). Assembly intracellular targeting and cell surface expression of the human *N*-methyl-D-aspartate receptor subunits NR1a and NR2A in transfected cells. *Neuropharmacology* **37**: 1355–1367.

McIlhinney RA, Philipps E, Le Bourdelles B, Grimwood S, Wafford K, Sandhu S. *et al* (2003). Assembly of *N*-methyl-D-aspartate (NMDA) receptors. *Biochem Soc Trans* **31**: 865–868.

Meador-Woodruff JH, Davis KL, Haroutunian V (2001a). Abnormal kainate receptor expression in prefrontal cortex in schizophrenia. *Neuropsychopharmacology* **24**: 545–552.

Meador-Woodruff JH, Hogg Jr AJ, Smith RE (2001b). Striatal ionotropic glutamate receptor expression in schizophrenia, bipolar disorder, and major depressive disorder. *Brain Res Bull* **55**: 631–640.

Meddows E, Le Bourdelles B, Grimwood S, Wafford K, Sandhu S, Whiting P *et al* (2001). Identification of molecular determinants that are important in the assembly of *N*-methyl-D-aspartate receptors. *J Biol Chem* **276**: 18795–18803.

Miller JA (1991). The calibration of 35S or 32P with 14C-labeled brain paste or 14C-plastic standards for quantitative autoradiography using LKB Ultrofilm or Amersham Hyperfilm. *Neurosci Lett* **121**: 211–214.

Molnar M, Potkin SG, Bunney WE, Jones EG (2003). MRNA expression patterns and distribution of white matter neurons in dorsolateral prefrontal cortex of depressed patients differ from those in schizophrenia patients. *Biol Psychiatry* **53**: 39–47.

Moy SS, Perez A, Koller BH, Duncan GE (2006). Amphetamine-induced disruption of prepulse inhibition in mice with reduced NMDA receptor function. *Brain Res* **1089**: 186–194.

Mueller HT, Meador-Woodruff JH (2004). NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder. *Schizophr Res* **71**: 361–370.

Nicolle MM, Baxter MG (2003). Glutamate receptor binding in the frontal cortex and dorsal striatum of aged rats with impaired attentional set-shifting. *Eur J Neurosci* **18**: 3335–3342.

Ohnuma T, Kato H, Arai H, Faull R, McKenna P, Emson P (2000). Gene expression of PSD95 in prefrontal cortex and hippocampus in schizophrenia. *Neuroreport* **11**: 3133–3137.

Perry EK, Perry RH, Tomlinson BE (1982). The influence of agonal status on some neurochemical activities of postmortem human brain tissue. *Neurosci Lett* **29**: 303–307.

Ransom RW, Stec NL (1988). Cooperative modulation of [3H]MK-801 binding to the *N*-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. *J Neurochem* **51**: 830–836.

Roche KW, Standley S, McCallum J, Dune Ly C, Ehlers MD, Wenthold RJ (2001). Molecular determinants of NMDA receptor internalization. *Nat Neurosci* **4**: 794–802.

Sans N, Prybylowski K, Petralia RS, Chang K, Wang YX, Racca C *et al* (2003). NMDA receptor trafficking through an interaction between PDZ proteins and the exocyst complex. *Nat Cell Biol* **5**: 520–530.

Sans N, Racca C, Petralia RS, Wang YX, McCallum J, Wenthold RJ (2001). Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway. *J Neurosci* **21**: 7506–7516.

Schipke CG, Ohlemeyer C, Matyash M, Nolte C, Kettenmann H, Kirchhoff F (2001). Astrocytes of the mouse neocortex express functional *N*-methyl-D-aspartate receptors. *FASEB J* **15**: 1270–1272.

Seifert G, Rehn L, Weber M, Steinhauser C (1997). AMPA receptor subunits expressed by single astrocytes in the juvenile mouse hippocampus. *Brain Res Mol Brain Res* **47**: 286–294.

Seifert G, Steinhauser C (1995). Glial cells in the mouse hippocampus express AMPA receptors with an intermediate Ca2+ permeability. *Eur J Neurosci* **7**: 1872–1881.

Shao Y, McCarthy KD (1997). Responses of Bergmann glia and granule neurons *in situ* to *N*-methyl-D-aspartate, norepinephrine, and high potassium. *J Neurochem* **68**: 2405–2411.

Shenolikar S (1995). Protein phosphatase regulation by endogenous inhibitors. *Semin Cancer Biol* **6**: 219–227.

Shoemaker JM, Saint Marie RL, Bongiovanni MJ, Neary AC, Tochen LS, Swerdlow NR (2005). Prefrontal D1 and ventral hippocampal *N*-methyl-D-aspartate regulation of startle gating in rats. *Neuroscience* **135**: 385–394.

Siegel BW, Sreekrishna K, Baron BM (1996). Binding of the radiolabeled glycine site antagonist [3H]MDL 105,519 to homomeric NMDA-NR1a receptors. *Eur J Pharmacol* **312**: 357–365.

Sills MA, Fagg G, Pozza M, Angst C, Brundish DE, Hurt SD *et al* (1991). [3H]CGP 39653: a new *N*-methyl-D-aspartate antagonist radioligand with low nanomolar affinity in rat brain. *Eur J Pharmacol* **192**: 19–24.

Sokolov BP (1998). Expression of NMDAR1, GluR1, GluR7, and KA1 glutamate receptor mRNAs is decreased in frontal cortex of 'neuroleptic-free' schizophrenics: evidence on reversible up-regulation by typical neuroleptics. *J Neurochem* **71**: 2454–2464.

Staiger JF, Flagmeyer I, Schubert D, Zilles K, Kotter R, Luhmann HJ (2004). Functional diversity of layer IV spiny neurons in rat somatosensory cortex: quantitative morphology of electrophysiologically characterized and biocytin labeled cells. *Cereb Cortex* **14**: 690–701.

Standley S, Roche KW, McCallum J, Sans N, Wenthold RJ (2000). PDZ domain suppression of an ER retention signal in NMDA receptor NR1 splice variants. *Neuron* **28**: 887–898.

Stratford KJ, Tarczy-Hornoch K, Martin KA, Bannister NJ, Jack JJ (1996). Excitatory synaptic inputs to spiny stellate cells in cat visual cortex. *Nature* **382**: 258–261.

Taylor GR, Carter GI, Crow TJ, Johnson JA, Fairbairn AF, Perry EK *et al* (1986). Recovery and measurement of specific RNA species from postmortem brain tissue: a general reduction in Alzheimer's disease detected by molecular hybridization. *Exp Mol Pathol* **44**: 111–116.

Terry-Lorenzo RT, Inoue M, Connor JH, Haystead TA, Armbruster BN, Gupta RP *et al* (2000). Neurofilament-L is a protein phosphatase-1-binding protein associated with neuronal plasma membrane and post-synaptic density. *J Biol Chem* **275**: 2439–2446.

Toro C, Deakin JF (2005). NMDA receptor subunit NRI and postsynaptic protein PSD-95 in hippocampus and orbitofrontal cortex in schizophrenia and mood disorder. *Schizophr Res* **80**: 323–330.

Torrey EF, Webster M, Knable M, Johnston N, Yolken RH (2000). The Stanley Foundation brain collection and neuropathology consortium. *Schizophr Res* **44**: 151–155.

Toyooka K, Iritani S, Makifuchi T, Shirakawa O, Kitamura N, Maeda K *et al* (2002). Selective reduction of a PDZ protein, SAP-97, in the prefrontal cortex of patients with chronic schizophrenia. *J Neurochem* **83**: 797–806.

Verkhratsky A, Kirchhoff F (2007). NMDA receptors in glia. *Neuroscientist* **13**: 28–37.

Verkhratsky A, Steinhauser C (2000). Ion channels in glial cells. *Brain Res Brain Res Rev* **32**: 380–412.

Wenthold RJ, Prybylowski K, Standley S, Sans N, Petralia RS (2003a). Trafficking of NMDA receptors. *Annu Rev Pharmacol Toxicol* **43**: 335–358.

Wenthold RJ, Sans N, Standley S, Prybylowski K, Petralia RS (2003b). Early events in the trafficking of *N*-methyl-D-aspartate (NMDA) receptors. *Biochem Soc Trans* **31**: 885–888.

White BH, Vogel MW (1996). CGP 39653 binding in the chick CNS after NMDA receptor antagonist treatment. *J Neural Transm* **103**: 1247–1253.

Woo TU, Walsh JP, Benes FM (2004). Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express the *N*-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. *Arch Gen Psychiatry* **61**: 649–657.

Yates CM, Butterworth J, Tennant MC, Gordon A (1990). Enzyme activities in relation to pH and lactate in postmortem brain in Alzheimer-type and other dementias. *J Neurochem* **55**: 1624–1630.

Ziak D, Chvatal A, Sykova E (1998). Glutamate-, kainate- and NMDA-evoked membrane currents in identified glial cells in rat spinal cord slice. *Physiol Res* **47**: 365–375.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)