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Abnormal Glutamate Receptor Expression in the Medial Temporal Lobe in Schizophrenia and Mood Disorders

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Pharmacological and anatomical evidence suggests that abnormal glutamate neurotransmission may be associated with the pathophysiology of schizophrenia and mood disorders. Medial temporal lobe structural alterations have been implicated in schizophrenia and to a lesser extent in mood disorders. To comprehensively examine the ionotropic glutamate receptors in these illnesses, we used *in situ* hybridization to determine transcript expression of *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and kainate receptor subunits in the medial temporal lobe of subjects with schizophrenia, bipolar disorder (BD), or major depression (MDD). We used receptor autoradiography to assess changes in glutamate receptor binding in the same subjects. Our results indicate that there are region- and disorder-specific abnormalities in the expression of ionotropic glutamate receptor subunits in schizophrenia and mood disorders. We did not find any changes in transcript expression in the hippocampus. In the entorhinal cortex, most changes in glutamate receptor expression were associated with BD, with decreased GluR2, GluR3, and GluR6 mRNA expression. In the perirhinal cortex we detected decreased expression of GluR5 in all three diagnoses, of GluR1, GluR3, NR2B in both BD and MDD, and decreased NR1 and NR2A in BD and MDD, respectively. Receptor binding showed NMDA receptor subsites particularly affected in the hippocampus, where MK801 binding was reduced in schizophrenia and BD, and MDL105,519 and CGP39653 binding were increased in BD and MDD, respectively. In the hippocampus AMPA and kainate binding were not changed. We found no changes in the entorhinal and perirhinal cortices. These data suggest that glutamate receptor expression is altered in the medial temporal lobe in schizophrenia and the mood disorders. We propose that disturbances in glutamate-mediated synaptic transmission in the medial temporal lobe are important factors in the pathophysiology of these severe psychiatric illnesses.
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INTRODUCTION

The hypothesis that glutamate is involved in the pathogenesis of severe mental illness was initially based in part on the effects of the hallucinogenic drug phencyclidine (PCP). PCP, and similar compounds that antagonize the *N*-methyl-D-aspartate (NMDA) receptor, can induce a schizophreniform psychosis in normal individuals and exacerbate these symptoms in schizophrenia (Hertzmann *et al*, 1990). Interestingly, other pharmacological studies and theoretical considerations have also implicated α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor dysfunction in schizophrenia. Positive modulators of the AMPA receptor, called ampakines, improve cognitive function in schizophrenia, whereas enhancement of AMPA receptor-mediated currents by these compounds may potentiate the activity of antipsychotic medications (Coyle, 1996). *In vitro* studies have demonstrated that NMDA and AMPA receptors colocalize in the postsynaptic membrane, acting synergistically at glutamatergic synapses (Kharazia *et al*, 1996).

The ionotropic AMPA, kainate, and NMDA glutamate receptors mediate most of the fast excitatory neurotransmission in the brain. AMPA receptors are ligand-gated channels that mediate the majority of low-frequency neurotransmission at glutamatergic synapses, and are heteromeric assemblies of GluR1–4 subunits. Kainate receptors are also ligand-gated ion channels composed of tetrameric combinations of five subunits, GluR5–7, KA1, and 2. Kainate receptors are located both post- and presynaptically, and influence the activity of other postsynaptic glutamate receptors by facilitating glutamate release and by modulating γ-aminobutyric acid (GABA)-

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ergic activity (Jiang *et al*, 2001). Variations in subunit composition or number of kainate receptors may therefore alter presynaptic glutamate release and/or the firing rate of NMDA and AMPA receptors.

NMDA receptors are tetrameric combinations of seven subunits, NR1, NR2A–D, NR3A, and 3B, with multiple binding sites including ones for glutamate, polyamine, Mg^{+2}, and glycine. The pharmacological regulation of the NMDA receptor depends on unique combinations of subunit-specific binding sites. The glycine (D-serine) co-agonist site must be occupied before glutamate activates the ion channel. Neurotransmission mediated by ionotropic glutamate receptors underlies several forms of synaptic plasticity in the hippocampus, including long-term potentiation (LTP) and depression (LTD) (Bliss and Collingridge, 1993; Isaac *et al*, 1995; Linden and Connor, 1995; Nicoll and Malenka, 1995; Selig *et al*, 1995; Bear and Abraham, 1996; Wu *et al*, 1996; Bear and Rittenhouse, 1999; Malenka and Nicoll, 1999). AMPA receptor colocalization with the NMDA receptor is required for LTP and other molecular correlates of learning, memory, and cognitive functions, highlighting the importance of ionotropic glutamate receptor function in the medial temporal lobe (Siegel *et al*, 1995).

The medial temporal lobe includes a system of anatomically related structures that are essential for establishing long-term declarative memory, likely due to their widespread and reciprocal connections with the neocortex. This system consists of the hippocampal region (CA subfields, dentate gyrus (DG), and subicular complex) and adjacent, anatomically related cortex, including entorhinal, perirhinal, and parahippocampal cortices. The entorhinal cortex, located in the ventromedial portion of the temporal lobe, has reciprocal connections with the hippocampus and other cortical and subcortical structures. The perirhinal cortex is a polymodal association area that contributes to recognition memory. Recent findings from lesion and electrophysiological studies suggest that this area participates in a broad range of memory functions, including both associative memory and emotional, as well as in consolidation functions. These results are consistent with neuroanatomical research showing that this area has strong reciprocal connections with widespread cortical sensory areas and with other memory-related structures, including the hippocampal formation and amygdala (Stefanacci *et al*, 1996; Suzuki, 1996; Wan *et al*, 1999).

Converging lines of evidence have implicated the medial temporal lobe in severe mental illness. Imaging approaches including magnetic resonance imaging (MRI) (Bogerts *et al*, 1990a, b; Lawrie and Abukmeil, 1998; Nelson *et al*, 1998; Lawrie *et al*, 1999; Velakoulis *et al*, 1999; Wright *et al*, 2000; Csernansky *et al*, 2002; Joyal *et al*, 2002; Pantelis *et al*, 2003), proton MRI (Maier *et al*, 1995; Bertolino *et al*, 1996, 1998; Deicken *et al*, 1999), and positron emission tomography (Friston *et al*, 1992; Tamminga *et al*, 1992; Medoff *et al*, 2001) have found abnormalities including decreased gray matter volumes in the medial temporal lobe in schizophrenia and bipolar disorder (BD). Neuropathology measures constitute the most substantial body of data for hippocampal involvement in serious mental illness, finding changes in neuronal size, density, and localization in the hippocampus in schizophrenia and BD, and to a lesser extent in major depressive disorder (MDD) (Arnold, 1997; Benes *et al*, 1998, 1999; Harrison, 1999; Weinberger, 1999; Harrison and Eastwood, 2001; Heckers and Konradi, 2002; Rajkowska, 2002a, b). Neurochemical studies suggest alterations in ionotropic glutamate receptor expression in schizophrenia, although contradictory data have been published (Gao *et al*, 2000; Law and Deakin, 2001). Taken together, these findings, and the role of glutamate as the major excitatory neurotransmitter in medial temporal structures, suggest that medial temporal lobe glutamatergic neurotransmission is compromised in schizophrenia and the mood disorders. We hypothesize that the expression of glutamate receptor subunits and/or binding of ionotropic glutamate receptors is altered in the medial temporal lobe in schizophrenia and the mood disorders, possibly reflecting an abnormal stoichiometry of the receptor.

MATERIALS AND METHODS

Subjects and Tissue Preparation

This study was performed using postmortem brains from 60 subjects obtained from the Stanley Foundation Neuropathology Consortium, consisting of four groups of 15 subjects, with diagnoses of schizophrenia, BD, MDD, and a comparison group (Torrey *et al*, 2000) (Table 1). These groups are matched for age, sex, race, postmortem interval (PMI), mRNA quality, brain pH, and hemisphere studied. Serial sections (14 μm) were provided by the Stanley Foundation and stored at −80°C.

In Situ Hybridization Histochemistry

Riboprobes were synthesized from linearized plasmids containing subclones of each of the ionotropic glutamate receptor subunits. Each probe recognizes all known isoforms and editing variants of each subunit (Table 2). In brief, 5 μl of [^{35}S]-UTP were dried and 2.0 μl 5X 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 T7 or Sp6 RNA polymerase were mixed and incubated for 2 h at 37°C. 1.0 μl DNase (RNase-free) was then added, and the mixture was incubated for 15 min at room temperature. The reaction mixture was loaded on spin columns (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 × SSC (standard saline citrate, 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 × SSC for 5 min and dehydrated in graded alcohols before air drying. [^{35}S]-labeled probes were applied diluting 3–5 × 10^6 c.p.m. in 400 μl per slide in hybridization buffer (50% deionized formamide, 3 × SSC, 1 × Denhardt's solution (0.02% polivinyl pyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin), 2% yeast tRNA (10 mg/ml), 50 mM sodium phosphate (pH 7.4), and 10%

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 (hours)	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

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

dextran sulfate in sterile water). Slides were covered with glass cover slips, and placed in humidified chambers containing blotter paper saturated with 50% formamide at 55°C overnight.

After 16 h of hybridization, cover slips were removed and sections were washed three times in 2 × 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 decreasing concentrations of SSC to a final stringency of 0.5 × SSC at 55°C for 1 h. Finally, the sections were dehydrated in increasing concentrations of ethanol, air dried, placed in X-ray cassettes, and apposed to Kodak Biomax MR film (Kodak, Rochester, NY) for 7–10 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

The conditions to determine binding to NMDA, AMPA, and kainate receptors have been previously described in detail (Healy *et al*, 1998; Healy and Meador-Woodruff, 1999; Ibrahim *et al*, 2000a, b), and used established receptor autoradiography assays methods (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). 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 (D-serine) coagonist site ([^{3}H]MDL105,519). [^{3}H]AMPA and [^{3}H]kainate were used to label those respective receptors. Slides from these studies were apposed 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), 2 weeks ([^{3}H]CGP39653), or 3 weeks ([^{3}H]AMPA and [^{3}H]kainate). Hyperfilm was developed in Kodak D-19 (4 min at 19°C), agitated in 2% acetic acid (30 s), fixed in Kodak Rapidfix (5 min), washed under running water (10 min) and air dried. Nonspecific binding was determined in adjacent slides from each subject by adding a specific blocker for each binding site ([^{3}H]MK-801: unlabeled MK-801; [^{3}H]ifenprodil: spermine; [^{3}H]CGP39653: glutamate; [^{3}H]MDL105,519: glycine; [^{3}H]AMPA and [^{3}H]kainate: CNQX) (Ibrahim *et al*, 2000a).

Image Analysis

Image analysis of in situ *hybridization films.* Developed films were digitized and quantitated using NIH image (v1.56) on a CCD-based imaging system. In the hippocampus, measurements were taken from the CA1, CA2, CA3, and CA4 subfields, DG, and subiculum (Sub). In the entorhinal and perirhinal cortices, each subunit had a distinct laminar distribution across the cortical thickness, and gray-scale values were obtained from discrete bands with density different from adjacent lamina (isodense bands). By overlapping images from Nissl counterstaining of the same slides we were able to correlate isodense bands to traditional cytoarchitectural layers. Gray scale values from hippocampal regions and cortical isodense bands were corrected for tissue background, and converted to optical density (OD). 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. Using the number of uridine nucleotides contained in each probe, the bound radioactivity value was converted to concentration of mRNA per hippocampal region or cortical isodense band, expressed as fmol/g of tissue. The values from two slides per subject were averaged to obtain a single value for each mRNA for each hippocampal region or cortical isodense band.

Table 2 Ionotropic Subunit Transcript Changes in Medial Temporal Lobe Structures

			Tukey HSD *post-hoc* test		
Area	**Transcript**	**Main effect for diagnosis**	**SZ**	**MDD**	**BD**
Hippocampus	NR1	$F=1.09$, df = 3, 283, $p=0.352$			
	NR2A	$F=2.79$, df = 3, 283, $p=0.041$			
	NR2B	$F=2.92$, df = 3, 292, $p=0.035$			
	NR2C	$F=3.09$, df = 3, 277, $p=0.028$			
	NR2D	$F=2.48$, df = 3, 280, $p=0.061$			
	GluR1	$F=3.03$, df = 3, 127, $p=0.032$			
	GluR2	$F=0.60$, df = 3, 274, $p=0.613$			
	GluR3	$F=0.64$, df = 3, 294, $p=0.589$			
	GluR4	$F=3.07$, df = 3, 282, $p=0.028$			
	GluR5	$F=2.61$, df = 3, 266, $p=0.052$			
	GluR6	$F=1.10$, df = 3, 304, $p=0.349$			
	GluR7	$F=2.01$, df = 3, 274, $p=0.112$			
	KA1	$F=2.51$, df = 3, 290, $p=0.059$			
	KA2	$F=2.26$, df = 3, 281, $p=0.082$			
Entorhinal	NR1	$F=3.38$, df = 3, 191, $p=0.019$			
	NR2A	$F=2.28$, df = 3, 216, $p=0.081$			
	NR2B	$F=2.90$, df = 3, 216, $p=0.036$			
	NR2C	$F=1.51$, df = 3, 216, $p=0.212$			
	NR2D	$F=2.91$, df = 3, 212, $p=0.036$			
	GluR1	$F=3.30$, df = 3, 378, $p=0.021$			
	GluR2	$F=9.41$, df = 3, 385, $p=0.000005$	nc	nc	$\downarrow p=0.00001$
	GluR3	$F=11.7$, df = 3, 378, $p=0.0000002$	nc	nc	$\downarrow p=0.0008$
	GluR4	$F=4.93$, df = 3, 378, $p=0.002$			
	GluR5	$F=1.07$, df = 3, 208, $p=0.361$			
	GluR6	$F=11.8$, df = 3, 384, $p=0.0000002$	nc	nc	$\downarrow p=0.00002$
	GluR7	$F=3.25$, df = 3, 204, $p=0.023$			
	KA1	$F=4.79$, df = 3, 200, $p=0.003$			
	KA2	$F=5.53$, df = 3, 250, $p=0.001$			
Perirhinal	NR1	$F=10.8$, df = 3, 311, $p=0.000001$	nc	nc	$\downarrow p=0.00007$
	NR2A	$F=6.44$, df = 3, 323, $p=0.0003$	nc	$\downarrow p=0.0004$	nc
	NR2B	$F=6.43$, df = 3, 318, $p=0.0003$	nc	$\downarrow p=0.005$	$\downarrow p=0.0005$
	NR2C	$F=5.62$, df = 3, 324, $p=0.0009$	nc	nc	nc
	NR2D	$F=1.09$, df = 3, 324, $p=0.355$			
	GluR1	$F=14.0$, df = 3, 312, $p=0.00000001$	nc	$\downarrow p=0.03$	$\downarrow p=0.000008$
	GluR2	$F=2.48$, df = 3, 323, $p=0.061$			
	GluR3	$F=6.77$, df = 3, 318, $p=0.0002$	nc	$\downarrow p=0.01$	$\downarrow p=0.0002$
	GluR4	$F=2.18$, df = 3, 312, $p=0.091$			
	GluR5	$F=5.61$, df = 3, 324, $p=0.0009$	$\downarrow p=0.028$	$\downarrow p=0.002$	$\downarrow p=0.003$
	GluR6	$F=2.93$, df = 3, 323, $p=0.034$			
	GluR7	$F=2.49$, df = 3, 323, $p=0.061$			
	KA1	$F=4.41$, df = 3, 318, $p=0.005$			
	KA2	$F=1.49$, df = 3, 317, $p=0.217$			

Image analysis of receptor autoradiography. Developed films were digitized and quantitated using the NIH imaging software program. For each hippocampal region and cortical isodense band, gray scale values from the 'total' binding were corrected for nonspecific binding from an adjacent slide for each subject, and absolute gray values were converted to OD. The amount of radioactivity bound (in nCi/g) was determined using [^{14}C]microscale standards

(Amersham Biosciences) (Miller, 1991), which were exposed on the same film as the slides for each study. From specific activity of each ligand, and percentage of receptor occupancy for the ligand (approximately 78% at three times K_d), values of bound radioactivity were converted to concentration of receptor binding per hippocampal region or cortical isodense band, expressed as pmol/g of tissue. The final value was averaged from two slides per subject.

Statistical Analysis

The dependent variable in the *in situ* hybridization studies was the mRNA concentration for each receptor subunit in each hippocampal region or cortical isodense band, whereas for receptor autoradiography the dependent variable was the concentration of receptor binding. For each dependent variable, correlation analysis was performed to test for associations between PMI, age, pH, and each dependent variable. If correlations were found ($\alpha = 0.05$) we used analysis of covariance (ANCOVA) to test the effect of diagnosis on mRNA or binding sites. When no significant correlations were detected, the effect of diagnosis on mRNA concentration and binding sites was determined by factorial analysis of variance (ANOVA), with diagnosis and isodense band or hippocampal subfield treated as independent variables for each area. We applied Bonferroni correction by which $\alpha = 0.001$. *Post hoc* analyses were performed using Tukey's honesty significant difference test and $\alpha = 0.05$ was used for significance (Tables 2 and 3, Supplementary Tables S4 and S5).

RESULTS

In Situ Hybridization

All glutamate ionotropic receptor subunit transcripts were detected in the medial temporal lobe structures examined. Sense strand riboprobes did not produce any specific labeling (data not shown).

Hippocampal Formation

NMDA subunit mRNAs. NMDA subunit mRNA expression was highest for NR1, followed by NR2B and NR2A; NR2C and NR2D were considerably lower in the hippocampus. All NMDA subunits were expressed in a similar distribution in hippocampal structures (Figure 1a–e). In the hippocampal formation, we found an association between NR1 mRNA expression and pH ($r = 0.55$, $p < 0.01$). We also found an association between pH and NR2A ($r = 0.53$, $p < 0.0001$) and NR2B ($r = 0.47$, $p < 0.001$). Using ANCOVA with pH as a covariate for NR1, NR2A, and NRB, we did not detect a main effect for diagnosis for these subunits. No association between NR2C and NR2D and pH, PMI, or age was found, and ANOVA did not detect a main effect of diagnosis the expression of these subunits (Table 2, Supplementary Figure S6).

AMPA subunit mRNAs. Similar to the NMDA receptor subunits, AMPA subunits were expressed in all hippocampal subregions (Figure 2a–d). We found an association between pH and GluR1 ($r = 0.38$, $p = 0.003$), GluR2 ($r = 0.43$, $p < 0.0005$), and GluR3 expression ($r = 0.43$, $p = 0.0005$). ANCOVA showed no main effect for diagnosis for any of these AMPA subunits. Using ANOVA, we also found no change of GluR4 expression (Table 2, Supplementary Figure S7).

Kainate subunit mRNAs. Kainate subunit transcripts were expressed in all hippocampal regions (Figure 3a–d). We found an association between GluR5 and pH ($r = 0.35$, $p = 0.006$), between GluR7 and PMI ($r = 0.26$, $p = 0.04$) and pH ($r = 0.47$, $p = 0.0002$), and between pH and both KA1 ($r = 0.43$, $p = 0.0008$) and KA2 ($r = 0.45$, $p = 0.0004$). ANCOVA using pH as a covariate for GluR5, KA1 and KA2, and PMI for GluR7, showed no significant changes in expression of these molecules. Likewise, ANOVA for GluR6 did not detect any effect of diagnosis (Table 2, Supplementary Figure S8).

Entorhinal Cortex

NMDA subunit mRNAs. All NMDA subunits showed four isodense bands of relatively higher intensity corresponding to cortical layers II, III, IV and V, and VI (Figure 1a–e). Regression analysis showed an association between NR1 and pH ($r = 0.33$, $p = 0.018$). ANCOVA using pH as covariate showed no main effect of diagnosis for NR1. Using ANOVA, we did not detect a main effect for diagnosis for any other NMDA subunits (Table 2, Supplementary Figure S9).

AMPA subunit mRNAs. In the entorhinal cortex, all AMPA receptor subunits were expressed in seven isodense bands (Figure 2a–d). There were no associations between PMI, pH, or age with any AMPA subunit transcript. Although no change was found for the expression of GluR1 and GluR4 subunits, ANOVA revealed a main effect for diagnosis for GluR2 ($F = 9.4$, $df = 3,385$, $p < 0.0001$) and GluR3 ($F = 11.7$, $df = 3,378$, $p < 0.0001$), with no diagnosis by layer interactions. *Post hoc* tests showed decreased GluR2 ($p < 0.0001$) and GluR3 expression ($p < 0.001$) in BD compared to controls (Table 2, Supplementary Figure S10).

Kainate subunit mRNAs. All kainate subunits were expressed in the entorhinal cortex, but with different distribution patterns. GluR5 had very low expression levels, followed by GluR6, KA1 and KA2 with moderate hybridization signal, and GluR7 with the strongest expression level in this cortical area (Figure 3a–e). We found an association between GluR6 expression and pH ($r = 0.3$, $p = 0.02$). ANCOVA revealed a main effect for diagnosis for GluR6 ($F = 11.82$, $df = 3,384$, $p < 0.0001$), with no diagnosis by isodense band interaction. *Post hoc* analysis showed decreased expression of this subunit ($p < 0.0001$) in BD. ANOVA revealed no diagnosis related changes of GluR5, GluR7, KA1, and KA2 expression (Table 2, Supplementary Figure S11).

Perirhinal Cortex

NMDA subunit mRNAs. In situ hybridization studies revealed a common distribution pattern for all NMDA receptor subunits consisting of six isodense bands (Figure 1a–e). Regression analysis of mRNA expression in the

Table 3 Ionotropic Receptor Binding Changes in Medial Temporal Lobe Structures

			Tukey HSD *post-hoc* test		
Area	**Ligand**	**Main effect for diagnosis**	**SZ**	**MDD**	**BD**
Hippocampus	MK801	$F=7.74$, $df=3, 582$, $p=0.00004$	↓ $p=0.0006$	nc	↓ $p=0.007$
	MDL105,519	$F=9.99$, $df=3, 631$, $p=0.000002$	nc	nc	↑ $p=0.008$
	Ifenprodil	$F=2.76$, $df=3, 610$, $p=0.042$			
	CGP39653	$F=7.02$, $df=3, 622$, $p=0.0001$	nc	↑ $p=0.0005$	nc
	AMPA	$F=0.35$, $df=3, 498$, $p=0.785$			
	Kainate	$F=3.00$, $df=3, 393$, $p=0.030$			
Entorhinal	MK801	$F=0.99$, $df=3, 107$, $p=0.400$			
	MDL105,519	$F=1.02$, $df=3, 112$, $p=0.386$			
	Ifenprodil	$F=1.64$, $df=3, 280$, $p=0.181$			
	CGP39653	$F=2.17$, $df=3, 110$, $p=0.096$			
	AMPA	$F=2.99$, $df=3, 250$, $p=0.032$			
	Kainate	$F=2.59$, $df=3, 275$, $p=0.053$			
Perirhinal	MK801	$F=2.18$, $df=3, 108$, $p=0.094$			
	MDL	$F=0.31$, $df=3, 110$, $p=0.821$			
	Ifenprodil	$F=1.88$, $df=3, 275$, $p=0.133$			
	CGP39653	$F=2.10$, $df=3, 162$, $p=0.102$			
	AMPA	$F=2.44$, $df=3, 110$, $p=0.068$			
	Kainate	$F=0.57$, $df=3, 275$, $p=0.635$			

SZ, schizophrenia; MDD, major depressive disorder; BD, bipolar disorder.

perirhinal cortex showed an association between pH and NR1 ($r=0.34$, $p=0.01$) and NR2A expression ($r=0.29$, $p=0.03$). Using ANCOVA, we detected a main effect for diagnosis for NR1 ($F=10.82$, $df=3,311$, $p<0.0001$) and NR2A ($F=6.44$, $df=3,323$, $p<0.001$). ANOVA for NR2B showed a main effect for diagnosis ($F=6.43$, $df=3,318$, $p<0.001$), but no diagnosis by isodense band interactions. *Post hoc* analysis revealed a significant decrease of NR1 in BD ($p<0.0001$), NR2A in MDD ($p<0.001$), and NR2B in both MDD ($p<0.01$) and BD ($p<0.001$) compared to controls. NR2C and NR2D were not changed (Table 2, Supplementary Figure S12).

AMPA subunit mRNAs. We found seven isodense bands for each of the AMPA receptor subunits detected in the perirhinal cortex (Figure 2a–d). We found an association between GluR2 and PMI ($r=0.30$, $p=0.02$). ANCOVA with PMI as covariate revealed no main effect for diagnosis for GluR2. ANOVA of GluR1, GluR3 and GluR4 expression showed a main effect for diagnosis for GluR1 ($F=13.97$, $df=3,312$, $p<0.0001$) and GluR3 ($F=6.77$, $df=3,318$, $p=0.0002$), but no changes for GluR4 expression. *Post hoc* analysis revealed a decreased GluR1 expression in MDD ($p=0.03$) and BD ($p<0.0001$) and decreased GluR3 expression in both mood disorders (MDD: $p=0.01$, BD: $p<0.001$) (Table 2, Supplementary Figure S13).

Kainate subunit mRNAs. Kainate receptor subunits showed a similar pattern of distribution to AMPA subunits in the perirhinal cortex, characterized by expression in six isodense bands (Figure 3a–e). Regression analysis showed an association between pH and expression of GluR7 ($r=0.387$, $p=0.003$). ANCOVA with pH as a covariate revealed no main effect for diagnosis for GluR7. ANOVA showed a main effect for diagnosis for GluR5 ($F=5.6$, $df=3,324$, $p<0.001$), with no diagnosis by layer interactions. *Post hoc* analysis showed decreased GluR5 expression in schizophrenia ($p=0.03$), MDD ($p=0.002$), and BD ($p=0.003$). No main effect for diagnosis was found for GluR6, KA1, or KA2 expression (Table 2, Supplementary Figure S14).

Receptor Binding Autoradiography

Specific binding of [^{3}H]AMPA, [^{3}H]kainate, and 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 all of the subdivisions of the hippocampus, and in entorhinal and perirhinal cortices (Figures 4a–h). In the hippocampus, receptor autoradiography revealed distribution patterns different from those seen from *in situ* hybridization studies of subunits transcripts. Less complex isodense banding patterns were found in the entorhinal and perirhinal cortices for receptor binding when compared to *in situ* hybridization (compare Figures 1 and 4). Both [^{3}H]AMPA and [^{3}H]kainate binding produced higher density bands in deep layers (V and VI) of the cortex, as well as in layer II (Figures 5a and c). Binding sites labeled by NMDA radioligands were more homogeneously expressed through cortical layers (Figure 4). [^{3}H]MDL105,519 produced the highest labeling of the NMDA ligands in

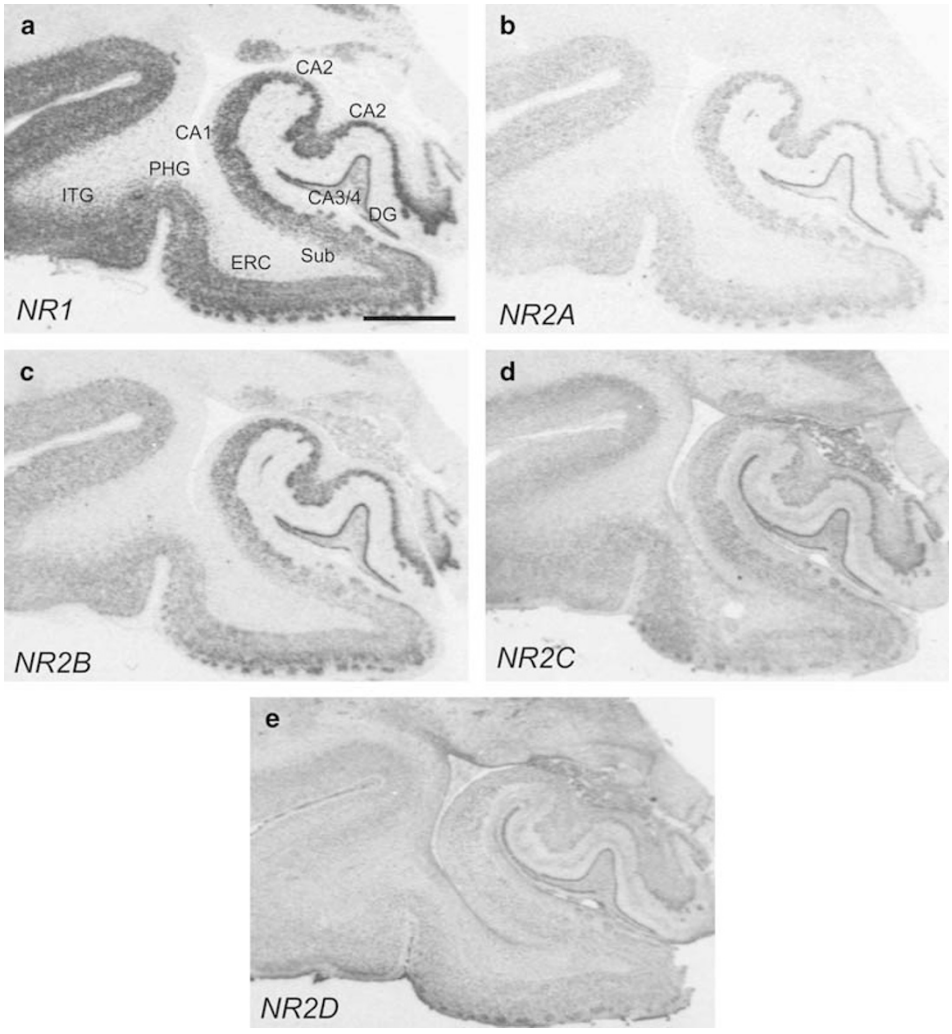

Figure 1 *In situ* hybridization using [^{35}S]-labeled antisense riboprobes for NMDA receptor subunit transcripts in the medial temporal lobe. CA1-4: Cornu Ammonis CA1-4, DG: dentate gyrus, Sub: subiculum, ERC: entorhinal cortex, PHG: parahippocampal gyrus, ITG: inferior temporal gyrus. Scale bar: 5 mm.

hippocampus, perirhinal and entorhinal cortex, follow by [^{3}H]MK801 (Figures 4e and g).

In the hippocampus, we found an association between [^{3}H]ifenprodil binding and PMI ($r = 0.4$, $p < 0.003$) and pH ($r = 0.3$, $p < 0.02$). Using ANCOVA with PMI and pH as covariates we did not detect a main effect for diagnosis for [^{3}H]ifenprodil binding. Analysis of variance for the other NMDA binding sites showed a main effect for diagnosis ([^{3}H]MK-801: $F = 7.74$, $df = 3,582$, $p < 0.0001$; [^{3}H]MDL105,519: $F = 9.99$, 3,631, $p < 0.0001$; [^{3}H]CGP39653: $F = 7,02$, $df = 3,622$, $p < 0.001$). *Post hoc* analysis revealed a significant reduction in the hippocampus of [^{3}H]MK-801 binding in schizophrenia ($p < 0.001$) and BD ($p < 0.001$), whereas [^{3}H]MDL105,519 binding was increased in BD ($p = 0.007$), and [^{3}H]CGP39653 increased in MDD ($p < 0.001$). In the hippocampus, we detected no association of kainate and AMPA receptor binding with PMI, pH, or age. Analysis of variance did not show a main effect for diagnosis for [^{3}H]kainate or [^{3}H]AMPA expression (Table 3, Supplementary Figure S15).

Receptor autoradiography analysis in the perirhinal and entorhinal cortices revealed no significant changes in the binding of any of the radioligands tested in schizophrenia or the mood disorders (Table 3, Supplementary Figures S16 and S17).

DISCUSSION

In this study, we found abnormalities in the expression of ionotropic glutamate receptor subunit transcripts in medial temporal cortex in schizophrenia and mood disorders. We observed striking differences between schizophrenia and the mood disorders. The subjects with schizophrenia showed few changes, whereas numerous alterations in the expression of glutamate receptor subunits were found for the mood disorders in the medial temporal cortex. No changes were found for any of the three illnesses in hippocampus, whereas the entorhinal cortex was only affected in BD. Both MDD and BD had robust expression changes of all three ionotropic receptor types in the perirhinal cortex. These results support our hypothesis of altered glutamatergic neurotransmission in the medial temporal lobe in schizophrenia and the mood disorders,

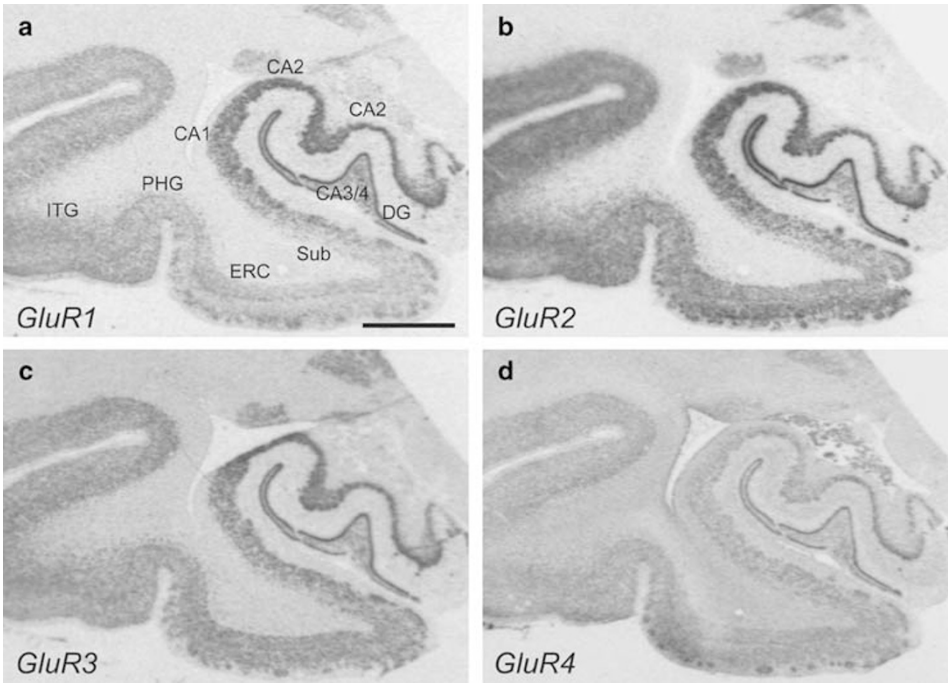

Figure 2 *In situ* hybridization using [^{35}S]-labeled antisense riboprobes for AMPA receptor subunit transcripts in the medial temporal lobe. See Figure 1 for abbreviations. Scale bar: 5 mm.

and highlight the specific regional distribution of the alterations of glutamate receptor subunit transcripts expression that occur in the mood disorders in the limbic cortex. Some of the results presented in this manuscript appear in a prior meta-analysis by the Stanley Neuropathology Consortium about the molecular abnormalities in the hippocampus in schizophrenia (Knable *et al*, 2004).

Altered Ionotropic Glutamate Receptor Expression in the Medial Temporal Lobe in Schizophrenia

The lack of changes in non-NMDA receptor subunits in the hippocampus contrasts with other reports that, although not including NMDA receptor subunit analysis, show that there are alterations in AMPA and kainate subunit transcripts, specifically decreased GluR1 and 2 (Eastwood *et al*, 1995, 1997), and GluR6 and KA2 (Porter *et al*, 1997) in the hippocampus in schizophrenia. (Harrison *et al*, 1991) reported decreased GluR1 mRNA expression in multiple hippocampal regions in schizophrenia (DG, CA3, CA4, and Sub), and decreased GluR2 transcripts in the parahippocampal gyrus. This group performed a second study of mRNA expression of GluR1 and GluR2 flip and flop isoforms, finding that both are decreased in hippocampal structures in schizophrenia (Eastwood *et al*, 1997). The inconsistencies between our results and these studies, as we did not find differences between regions of the hippocampus, nor any AMPA or kainate expression changes in schizophrenia, might be due to differences in the subject cohorts, or to methodological reasons, such as differences between RT-PCR *vs in situ* hybridization.

However, we detected changes in [^{3}H]MK801 binding in the hippocampus in schizophrenia. Interestingly, a recent study found that the effects of PCP administration on the firing pattern of neurons in the prefrontal cortex are mediated by afferents from the hippocampus. Studies show that PCP-induced long-lasting activation of the prefrontal cortex plays a pivotal role in the development of behavioral abnormalities comparable to some symptoms of schizophrenia (Moghaddam and Adams, 1998; Suzuki *et al*, 2002; Jodo *et al*, 2003; Takahata and Moghaddam, 2003). A recent study identified the hippocampus as a source of PCP-induced excitatory inputs to the prefrontal cortex by a dense glutamatergic projection to the medial prefrontal cortex (Jay *et al*, 1992; Takahata and Moghaddam, 2003) originated in the ventral hippocampus. Disinhibitory activation of CA1 pyramidal cells can be produced by inhibition of tonic GABA inputs by NMDA antagonists like PCP (Gurguis *et al*, 1990; Jay *et al*, 1992). PCP locally infused into the ventral hippocampus increased spontaneous discharges of prefrontal neurons with augmentation of locomotor activity in freely moving rats. Local application of a more selective NMDA receptor antagonist, MK801, to ventral hippocampal neurons under anesthesia increased the spontaneous firing rates of most neurons directly projecting to the medial prefrontal cortex, whereas local application of MK801 to medial prefrontal cortical neurons did not induce excitatory responses in any of those neurons. This suggests that the behavioral abnormalities in schizophrenia might be related to a malfunction of the prefrontal cortex as a response to a change in glutamate neurotransmission in the hippocampus, related to an alteration of NMDA receptors in the postsynaptic hippocampus cells, which is consistent with our present results of decreased MK801 binding in the hippocampus.

Noteworthy, changes in glutamate receptor subunit expression in the temporal cortex in schizophrenia in our current work were also very limited restricted to decreased

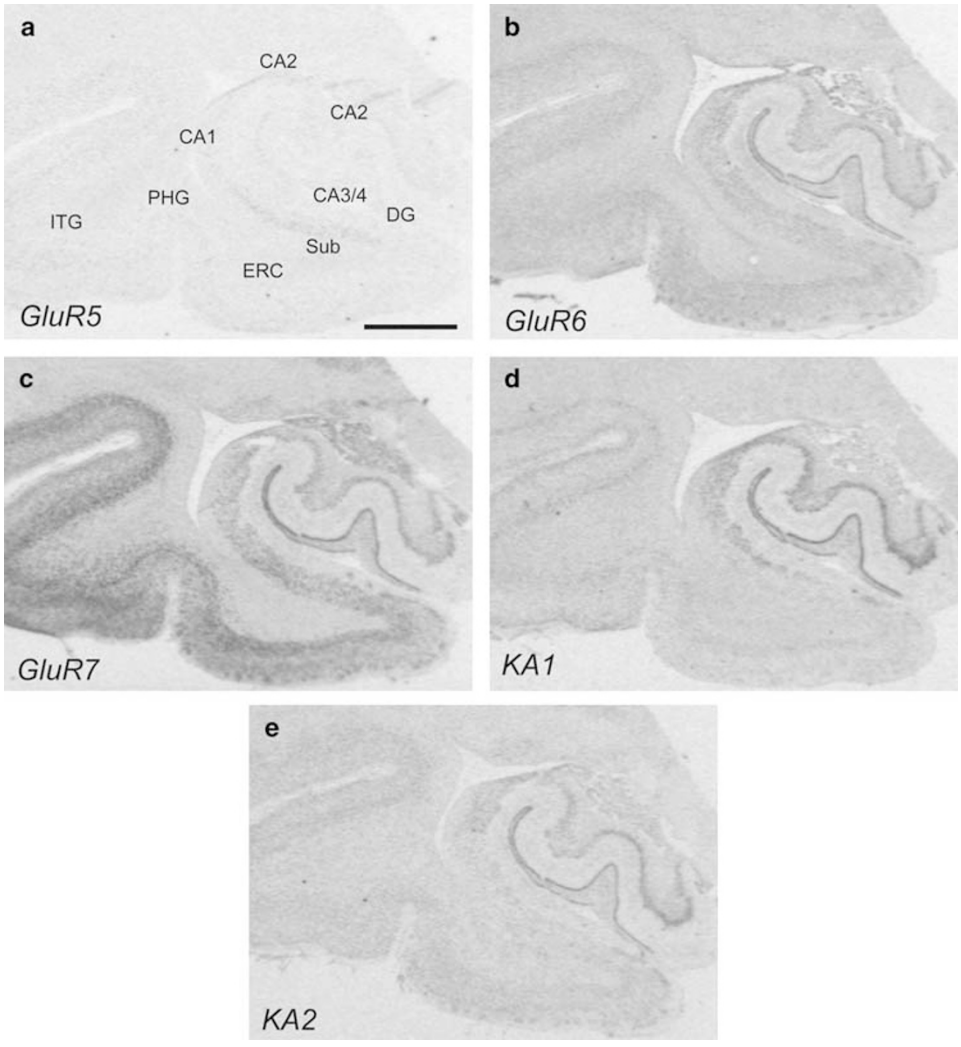

Figure 3 *In situ* hybridization using [^{35}S]-labeled antisense riboprobes for kainate receptor subunit transcripts in the medial temporal lobe. See Figure 1 for abbreviations. Scale bar: 5 mm.

GluR5 subunit expression in the perirhinal cortex. These data correlate with reports on the expression of this subunit in the dorsolateral prefrontal cortex in schizophrenia by Scarr *et al* (2005).

Altered Ionotropic Glutamate Receptor Expression in the Medial Temporal Lobe in MDD

Recent findings indicate that dysfunction of glutamatergic pathways in the brain may be one of the mechanisms involved in the pathophysiology of depression (Heresco-Levy and Javitt, 1998). In addition, deficits of NMDA receptors have been found in the frontal cortex of suicide victims (Nowak *et al*, 1995, 2003). In MDD, we found robust changes in the expression of several key glutamate receptor subunits in the perirhinal cortex. Among the NMDA receptor subunits, expression of NR2A and NR2B transcripts were drastically reduced.

The differential expression of NR2 subunits during brain development has been proposed to reflect not only maturational changes, but to have physiological roles in the nervous system (Komuro and Rakic, 1993; Sucher *et al*, 1995; Behar *et al*, 1999; Ikonomidou *et al*, 1999). Depending on their subunit composition, NMDA receptors have different electrophysiological properties (Monyer *et al*, 1994; Sucher *et al*, 1995). For example, receptors composed of NR1/NR2A subunits show the fastest and NR1/NR2D show the slowest deactivation kinetics. The Mg^{2+} blockade is different among receptors with different subunit composition, where only NMDA receptors containing NR2A or NR2B subunits are blocked. In our results in the perirhinal cortex, we detected decreased expression of these two subunits in MDD, suggesting a reduction in the probability of Mg^{2+} blockade of NMDA receptors.

Recently, some studies have focused on the importance of NMDA receptor subunit composition and signaling at the postsynaptic cell. Biochemical studies have shown high-affinity binding between the catalytic domain of CaMKII and the C-tail of NR2B subunit of the NMDA receptor (Strack and Colbran, 1998; Leonard *et al*, 1999; Strack *et al*, 2000; Bayer *et al*, 2001; Mayadevi *et al*, 2002). Many brain regions, including the hippocampus, show changes from

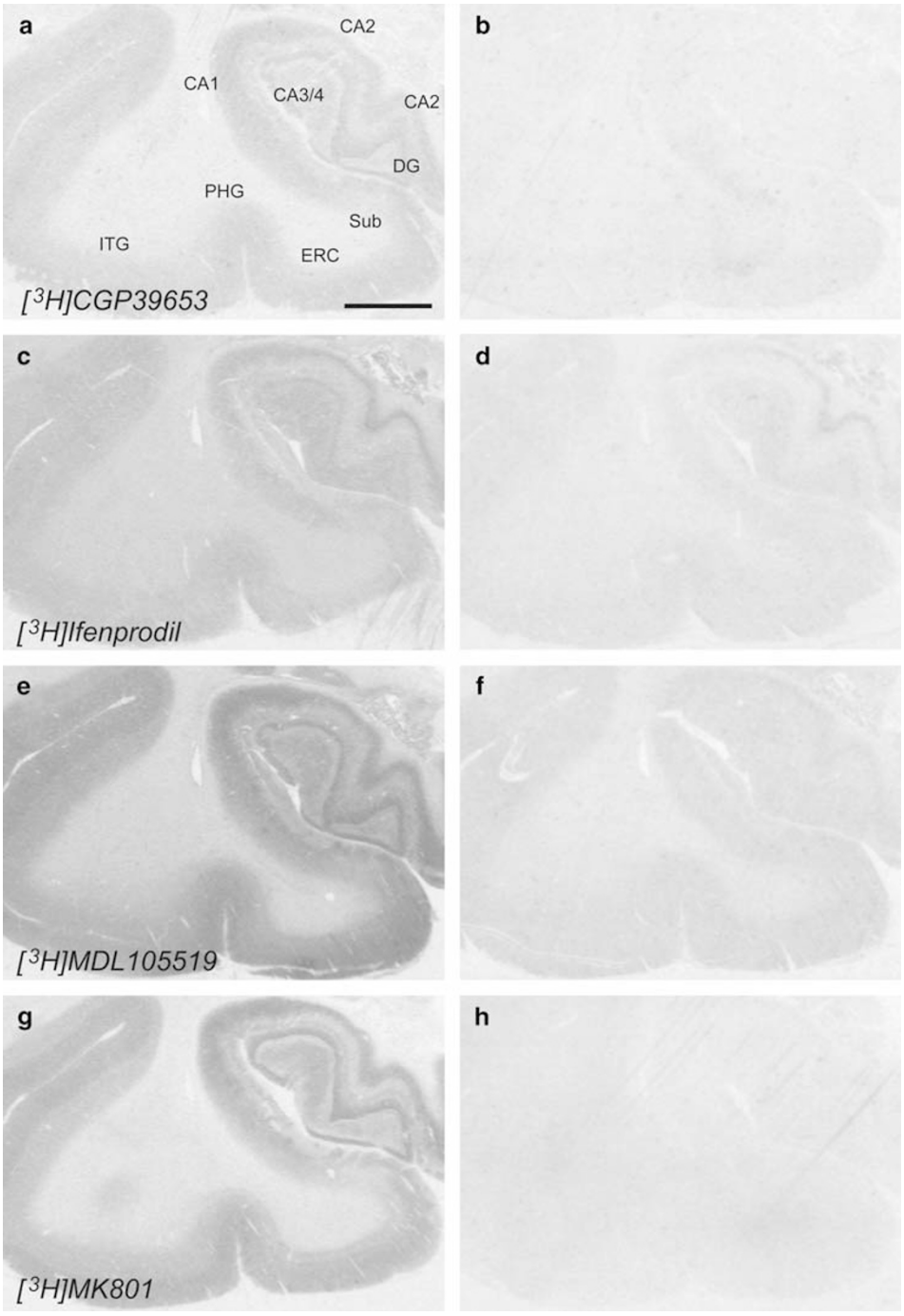

Figure 4 NMDA receptor autoradiography in the medial temporal lobe. b, d, f, and h: non-specific binding in the presence of blockers for each ligand. See Figure 1 for abbreviations. Scale bar: 5 mm.

containing predominantly NR2B to containing NR2A in response to different stimuli (Monyer *et al*, 1994; Sheng *et al*, 1994). This change can be driven by activity (Barria and Malinow, 2002), experience (Quinlan *et al*, 1999; Philpot *et al*, 2001; Mierau *et al*, 2004), or learning (Quinlan *et al*, 2004). In mature animals, some brain regions (eg, the hippocampus) contain predominantly NR2A, and both NR2A and NR2B NMDA receptor subunits play important roles in LTP (Sakimura *et al*, 1995; Ito *et al*, 1996; Kim *et al*, 2005). Interestingly, it has been shown that acute replacement of synaptic NR2B with NR2A decreases LTP and spontaneous activity-driven potentiation characterized by a drive of AMPA receptor into synapses (Zhu and Malinow, 2002; Barria and Malinow, 2005), as do mutations of NR2B that prevent association to active CaMKII (Barria and Malinow, 2005). An association between active CaMKII and NR2B has been shown for some forms of activity-driven synaptic potentiation by AMPA receptor trafficking to the membrane. Our results of lower expression of both NR2A and NR2B transcripts in MDD suggest a possible compro-

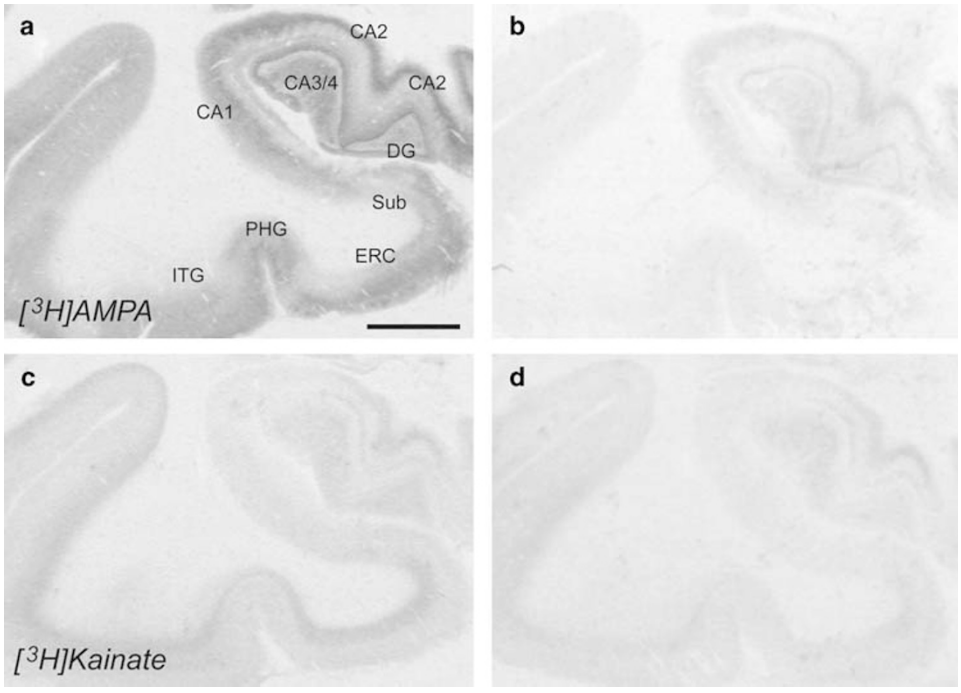

Figure 5 AMPA and kainate receptor autoradiography in the medial temporal lobe. b and d: non-specific [^{3}H]AMPA and [^{3}H]kainate binding in the presence of CNQX. See Figure 1 for abbreviations. Scale bar: 5 mm.

mise in LTP and, consequently, in AMPA receptor trafficking to hippocampal synapses. Studies of activation of CaMKII and trafficking of AMPA receptors in the hippocampus in MDD would be needed to support that hypothesis.

GluR1 and GluR3 AMPA receptors also showed lower expression in MDD compared to controls. These two subunits bind to specific intracellular proteins that mediate two of the AMPA trafficking pathways at the postsynaptic density. It has been suggested that GluR1 is the subunit providing the 'driving force' for activity-dependent synaptic delivery of AMPA receptors, whereas GluR2/3 mediate the constitutive recycling and regulated endocytosis of AMPA receptors. SAP97 is required for the trafficking of newly synthesized GluR1-containing AMPA receptors in response to increased synaptic activation. The decreased expression of GluR1 in the perirhinal cortex in MDD could compromise the fast trafficking of AMPA receptors from the Golgi to the postsynaptic membrane in cellular mechanisms like LTP (Esteban, 2003). The downregulation of GluR3 also suggests that the rapid recycling and/or storage in the trafficking pathway involving GluR2/3 might be disrupted in MDD.

Abnormal Ionotropic Glutamate Receptor Expression in the Medial Temporal Lobe in BD

As in MDD, no alterations in mRNA expression in the hippocampus were seen in BD. However, changes in glutamate receptor expression in medial temporal cortices were found, and contrasting with MDD, changes were also present in the entorhinal cortex. GluR1 transcripts were downregulated in the perirhinal cortex but not in the entorhinal, GluR2 reduced only in the entorhinal, whereas GluR3 had reduced expression in both areas. As explained before for the changes found in MDD, alterations on the expression of AMPA subunits suggest that trafficking of the AMPA receptors mediated by them. Abnormal trafficking of AMPA receptors towards the cell membrane could affect activation of NMDA receptors. In this case, we also found a decrease in GluR2 expression maybe as a compensatory effect to overcome the lower concentration of intracellular Ca^{+2} owing to the lower activation of NMDA receptors. This hypothesis is also supported by the reduction in the expression of GluR3 in both cortical areas, as GluR3 mediates the recycling of AMPA receptors regulated by NMDA activation.

Similar to our finding in schizophrenia, in BD we found a significant decrease in [^{3}H]MK-801 binding in the hippocampus, consistent with a recent report that also found a significant decrease in [^{3}H]MK-801 binding in CA3 and the Sub (Scarr *et al*, 2003). As described above, this finding suggests an alteration in the quantity and activity of NMDA receptors in hippocampal neurons that could impact hippocampal connectivity with the prefrontal cortex and might contribute to behavioral abnormalities in this illness. We also found an increase in [^{3}H]MDL105,519 binding in the hippocampus in BD, indicating an increase in the number of NMDA-receptor containing glycine binding sites in this region. The MK-801 and glycine NMDA receptor binding sites preferentially bind NR2A-B and NR1-containing NMDA receptors, respectively (Lynch *et al*, 1994). However, we did not find any changes in NR1, NR2A, or NR2B transcript expression in the hippocampus in BD, results that are inconsistent with either a change in NMDA receptor stoichiometry or a change in the total number of

receptors. One explanation for these divergent results is that there are changes in NMDA receptor subunit protein levels that are not reflected at the level of transcript expression. Alternatively, there may be divergent changes in subsets of neurons expressing NMDA receptor subunits and binding sites. As our experimental techniques only permit regional level analyses, we would not have detected changes in expression in different subsets of cells.

The entorhinal cortex is a crucial component of the medial temporal lobe memory system (Scoville and Milner, 1957; Squire and Zola-Morgan, 1991). Layer V neurons of the entorhinal cortex receive convergent sensory input from other cortical areas (Room and Groenewegen, 1986; Rempel-Clower and Barbas, 2000), give rise to a massive projection to the cerebral cortex, including the dorsolateral prefrontal cortex (Insausti *et al*, 1997), and are the target of hippocampal output (Nussenzveig *et al*, 1991). The changes we detected in the entorhinal and perirhinal cortices might represent a disruption in glutamatergic neurotransmission in these cortical areas as postsynaptic targets of prefrontal or hippocampal input, or of transcripts destined to presynaptic terminals from the projections they send back to the cortex or hippocampus.

Limitations of the Study

Several limitations need to be considered when interpreting data from these studies. First, an important limitation of this and all postmortem studies in psychiatric illness is the possible confounding effect of psychotropic medications. The majority of patients with schizophrenia and mood disorders from this study were exposed to psychotropic drugs at some point in life (Torrey *et al*, 2000). Antipsychotic medications can modulate early gene expression (Deutch *et al*, 1995; Cohen and Wan, 1996; Holcomb *et al*, 1996). They do not, however, appear to affect thalamic NMDA receptor expression (Ulas *et al*, 1993). Dracheva *et al* (2001) reported altered NMDA receptor subunit expression in the prefrontal and occipital cortices 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 (Dracheva *et al*, 2001). Less is known about the effects of antidepressants and mood stabilizers on the expression of glutamate receptors. 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). Although 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.

Another limitation of this study is that our receptor subunit transcript expression data and our receptor binding data are not consistent with one another. As changes in binding site expression generally reflect changes in receptor subunit stoichiometry, one would expect to find some changes in subunit mRNA expression in instances where there are changes in binding site expression. Possible explanations for these discrepancies include (1) we did not have sufficient power to detect small changes in mRNA expression, or (2) changes in receptor subunit expression are not evident at the transcript level, but might be detected at the protein level. The protein levels are determined not only by the level of the mRNA that codifies it and its translation rate, but also by the folding efficiency of the protein itself, posttranslational modifications, binding with other components to form protein complexes, and the rate of protein degradation. Some of these events could modify protein levels without a change in mRNA expression (You and Yin, 2000; Kristiansen *et al*, 2006).

In summary, our data support that in schizophrenia and mood disorders, glutamate receptor expression is altered in the medial temporal lobe. However, all three illnesses, but most remarkably schizophrenia and BD, have a unique distribution of glutamatergic alterations. Although we propose that compromised glutamate-mediated synaptic neurotransmission in these limbic areas might be an important component of the pathophysiology of schizophrenia and the mood disorders, the regional heterogeneity of these alterations might explain the dissimilarity of symptoms between schizophrenia and mood disorders. Although glutamate abnormalities in schizophrenia are characterized for their major neocortical, thalamic, and striatal components (some also shared by BD), the present data suggest that glutamatergic abnormalities in BD can be further typified by a parahippocampal cortical location (entorhinal and perirhinal cortices), previously suggested to be responsible for the deficits found in working memory and attention (Adler *et al*, 2004).

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