Original Article

Antibodies to the glutamate receptor in mania


Background: There is evidence that the glutamatergic system is involved in the pathophysiology of mania. Antibodies to the NR2 subunits of the N-methyl-D-aspartate (NMDA) receptor have been shown to adversely affect glutamate functioning.

Methods: We measured serum antibodies to the NR2 peptide of the NMDA receptor in 60 individuals with different subtypes of mania, including schizoaffective cases, who were assessed at up to three time points. We also measured these antibodies in 295 individuals in other psychiatric groups and in 170 non-psychiatric controls. NR2 antibody levels were compared among groups by multivariate analyses and within the mania group by repeated measures analysis of variance.

Results: Individuals with mania had increased levels of antibodies to the NR2 peptide compared to levels in non-psychiatric controls when measured at the time of admission ($t = 2.99$, $p = 0.003$) and the time of evaluation ($t = 2.57$, $p = 0.010$), but not at follow-up six months later. The levels of antibodies in individuals in other psychiatric groups did not differ significantly from the levels measured in the control population. Within the mania group, there was a significant decrease in antibody levels over the three time points of the study ($F = 5.4$, $df = 2$, $p = 0.0067$).

Conclusions: NR2 antibodies are elevated during the acute phase of mania but not at follow-up. Our findings support a role for antibodies to the NMDA receptor in the pathogenesis of acute mania.

Mania is an abnormal mood state and the defining characteristic of bipolar disorder. The etiology of mania is largely unknown. Although genetic factors play a major role in the etiology of bipolar disorder, most have relatively low odds ratios (ORs) (1). Immunological abnormalities have been identified and may contribute to the pathophysiology of mania as well as to bipolar disorder more broadly (2–6). Such factors may help to explain the marked fluctuations in mood which are the hallmark of the disorder.

The N-methyl-D-aspartate (NMDA) receptor complex, part of the glutamatergic system, plays an important role in the regulation of neuronal communication and synaptic function in the central nervous system (CNS) (7). There is evidence that the glutamatergic system is involved in the pathophysiology and treatment of mania (8). The NMDA receptor is present in the brain primarily as a tetramer and is a target of the protein kinase C (PKC) signaling cascade. Overactive PKC signaling in the prefrontal cortex is thought to explain many of the symptoms of mania (9). In addition, lithium, a major treatment for mania, exerts major effects on the PKC signaling cascade (8, 10). Further evidence for the involvement of the NMDA complex in mania and in bipolar disorder comes from postmortem studies which have found altered NMDA–receptor complexes in the brain tissue of persons with bipolar disorder (11, 12).

The NR2 subunits of the NMDA receptor contain the binding site for glutamate. NR2 subunits are expressed differentially across various cell types and control the electrophysiological properties of the NMDA receptor. Recently, autoantibodies to the NR2 receptor have been...
identified in the blood and cerebrospinal fluid of some individuals. These antibodies to NR2 are generally associated with tissue damage or an immune response within the CNS (13). Dalmau and colleagues have described anti-NMDA encephalitis as a multi-stage illness that presents initially with psychotic symptoms, seizures, and cognitive problems and proceeds to catatonia and coma; in spite of the severity of the syndrome, most patients make a substantial or full recovery (14, 15). There is also a single case report of antibodies to the NR2 subunit in the blood and cerebrospinal fluid (CSF) of an adult woman with acute mania who also had symptoms of encephalitis (16). In another recent case report, an adolescent male was described with an acute onset of severe neurological and psychotic symptoms; he was found to have elevated levels of antibodies to the NR1/NR2 subunits of the NMDA receptor in his serum and CSF and was diagnosed with anti-NMDA encephalitis (17). However, NR2 antibodies have not been previously examined in populations of people with mania or other psychiatric disorders.

In this study we examined the levels of serum antibodies to the NR2 peptide fragment of the NMDA receptor in individuals hospitalized for mania at three time points. The assay that we used measured antibodies to all four of the NR2 receptor types as well as the closely related NR1 receptor. We also compared the levels in the mania samples with those of other psychiatric study populations and with non-psychiatric controls and examined whether individuals with mania had increased levels of antibodies to other human antigens.

**Patients and methods**

**Participant recruitment and characterization**

The study sample consisted of 525 individuals in five groups: 60 hospitalized for symptoms of mania; 55 with a recent onset of psychosis; 180 with multi-episode schizophrenia; 60 with bipolar disorder not selected for mania; and 170 controls otherwise specified. In contrast to all of the patients in the bipolar disorder sample was evaluated during a hospital admission for mania. Individuals without a history of psychiatric disorder were recruited from posted announcements at local health care facilities and universities in the same geographic area as the settings where the psychiatric participants were recruited. The control individuals were enrolled after they had been screened to rule out the presence of a current or past psychiatric disorder using the Structured Clinical Interview for DSM-IV (SCID) Axis I Disorders – Non-patient edition (18). Participants in all studies met the following additional criteria: age 18–65 (except the recent-onset patients, who were aged 18–45); proficient in English; absence of any history of intravenous substance abuse; absence of mental retardation; absence of HIV infection; absence of serious medical disorder that would affect cognitive functioning; and absence of a primary diagnosis of alcohol or substance use disorder. For the purposes of the study sample, participants were only included in one psychiatric group with the order of selection: mania > recent onset psychosis > multi-episode schizophrenia or bipolar disorder not selected for mania. The diagnosis of the psychiatric participants was established by consensus of the research team based on
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The SCID for DSM-IV Axis I Disorders – Patient Edition (19) and available medical records.

The studies were approved by the Institutional Review Boards of the Sheppard Pratt Health System and the Johns Hopkins Medical Institutions following established guidelines. All participants provided written informed consent after the study procedures had been explained.

Participants were asked about their educational level and other demographic variables as well as maternal education as a proxy for pre-morbid socioeconomic status. Data were obtained about current height and weight, from which body mass index (BMI) was calculated, and current smoking status. All participants were individually administered a brief cognitive battery, the Repeatable Battery for the Assessment of Neuropsychological Status, Form A (RBANS) (20) at the baseline visit. All of the psychiatric participants were also interviewed and rated on the Positive and Negative Syndrome Scale (PANSS) (21) and the mania patients were rated on mood rating scales including the Young Mania Rating Scale (YMRS) (22). For the mania patients, this clinical evaluation took place at the time of the baseline visit during the hospital stay and at the six-month follow-up. For all of the psychiatric participants, psychiatric medication data were recorded from participant self-report and clinical charts and it was noted whether each patient was receiving each of the following types of medication at the time of the study visits: lithium, anticonvulsant mood stabilizer, atypical antipsychotic agent, antidepressant. At the follow-up visit, mania participants were also asked about their interval treatment history, including the occurrence and dates of any psychiatric hospitalization during the follow-up period, which were verified when possible from hospital records.

Laboratory evaluations

A blood sample was obtained at the study visit for those with recent onset of psychosis, multi-episode schizophrenia, bipolar disorder not selected for mania, and control participants. A blood sample was obtained at three time points for most mania participants: from the day of hospital admission from archived samples that were collected as part of the admission medical work-up; at the time of consent to the study and baseline evaluation during the hospital stay; and at the time of the six-month follow-up. In all samples, serum antibodies to the NR2 peptide fragment of the NMDA receptor were measured by the Gold Dot NR2, which is a serological enzyme-linked immunosorbent assay for the quantitative determination of immunoglobulin (Ig) G class antibodies to the NR2 subunit of the human NMDA glutamate receptor in the serum (http://www.cisbiotech.com/media/FlyerAB_GoldDotNR2_s.pdf). This assay measures antibodies to an immunoactive NR2A/2B peptide which is 21 amino acids in length, corresponding to the N-terminal sequence of the NMDA receptor (23). IgG class antibodies to double-stranded DNA and to tissue transglutaminase were measured by enzyme immunoassay using standard methodologies.

Data analyses

The clinical and demographic characteristics of persons in the five diagnostic groups were compared by one-way analysis of variance (ANOVA) or chi-square analysis. Mania participants in the admission group and in the follow-up group were compared to mania participants not seen at each of these visits. ANOVA was used to compare the levels of antibodies to NR2 among the three mania samples (at the time points of admission, baseline evaluation, and follow-up). Linear regression models with robust standard errors were used to compare the levels of the antibodies among all seven study groups (mania at three time points, recent onset psychosis, multi-episode schizophrenia, bipolar disorder not selected for mania, and controls), adjusting for age, gender, race, and maternal education. Robust standard errors and cluster analysis were used to control for the multiple measurements among the individuals with mania.

Logistic regression models were used to calculate the ORs associated with increased levels of antibodies in each of the psychiatric study groups; increased levels of antibodies were defined as antibody levels ≥ 90th percentile of the controls. In these models we adjusted for age, gender, race, and maternal education. Within the mania group, repeated measures ANOVA was used to determine the association between change in the level of NR2 antibodies over the time points of the study (admission, evaluation, follow-up). Within the mania group, repeated measures ANOVA was also used to determine if the levels of antibodies to double-stranded DNA or to tissue transglutaminase differed over time. For the psychiatric participants as a group, one-way ANOVA was used to determine if NR2 antibody levels were associated with receipt of the following medications: lithium, anticonvulsant, antidepressant, and antipsychotic medications.

All statistical analyses were performed using STATA version 11 (College Station, TX, USA).
Results

The demographic and clinical characteristics of the study populations are presented in Table 1. The five study groups differed significantly \( p < 0.05 \) on all of the demographic and clinical variables, including age, race, gender, education, maternal education, BMI, current smoking status, RBANS cognitive score, and, among the patient groups, on PANSS positive, negative, general, and total symptom scores. The medications that psychiatric participants were receiving at the time of the study evaluations are shown in Table 2.

All 60 mania patients were seen at the baseline evaluation visit. A total of 44 out of 60 (73%) also had a hospital admission blood sample, which was drawn on average 4.0 \( \pm 2.3 \) days before the evaluation sample. Follow-up contact was attempted for all of the participants seen at the baseline evaluation visit per the study protocol. A total of 39 out of 60 (65%) mania participants were evaluated at a six-month follow-up visit and had a blood sample drawn at that time. The mean time between enrollment and follow-up was 191.7 days \( \pm 13.3 \) days. The mean YMRS score at the baseline evaluation was 18.4 \( \pm 8.4 \) and at the six-month follow-up was 7.3 \( \pm 6.8 \); it is of note that the baseline evaluation was performed several days after hospital admission, so may not reflect patient acuity at the time of admission. Of the 21 mania participants not seen for a follow-up visit, three refused and 18 could not be located. A total of 29 (48%) of the mania participants had samples from all three time points. The mania participants who were evaluated at hospital admission and at the six-month follow-up did not differ from those who were not evaluated at these time points in their baseline

<table>
<thead>
<tr>
<th>Table 1. Demographic and clinical characteristics of study populations</th>
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<tr>
<td><strong>Mania</strong> (n = 60)</td>
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<tr>
<td>Age, years, mean (SD)</td>
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<tr>
<td>Gender, male, n (%)</td>
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<tr>
<td>Race, Caucasian, n (%)</td>
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<td>Education, years, mean (SD)</td>
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<td>Maternal education, years, mean (SD)</td>
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<td>BMI, mean (SD)</td>
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<td>Current cigarette smoker, n (%)</td>
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<td>RBANS cognitive score, mean (SD)</td>
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BMI = body mass index; PANSS = Positive and Negative Syndrome Scale; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status, Form A; SD = standard deviation.

*The mania group differed significantly from the control group only on the RBANS cognitive score; \( F = 43.17, p < 0.01 \).

**Significant difference among groups in one-way analysis of variance \( p < 0.05 \).

*Schizophrenia: n = 177; controls: n = 168.

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<tr>
<th>Table 2. Medications received by psychiatric study participants*</th>
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<tr>
<td><strong>Mania at baseline</strong> (n = 60)</td>
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<tr>
<td>Atypical antipsychotic agents, n (%)</td>
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<tr>
<td>Lithium, n (%)</td>
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<tr>
<td>Anticonvulsants, n (%)</td>
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<td>Antidepressants, n (%)</td>
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*The totals in each column are > 100% because many patients received more than one type of medication.

**A total of four patients (10%) were not receiving any psychiatric medications.
PANSS symptom scores, YMRS score, age, maternal education, education, gender, race, or receipt of a specific class of medication (all p > 0.05).

The levels of NR2 antibodies measured in the individuals in the different study groups are depicted in Figure 1. Individuals with mania had increased levels of antibodies as compared to controls when measured at the time of hospital admission (t = 3.00, p = 0.003) and the time of evaluation (t = 2.57, p = 0.011), which was on average 6.1 days following admission. The levels of antibodies measured in individuals with mania at follow-up did not differ significantly from those in controls (t = 0.44, p = 0.664). A decrease in antibody levels over time was also confirmed by repeated measures ANOVA, which indicated a significant interaction between antibody level and time of evaluation (F = 5.4, df = 2, p = 0.0067).

By contrast, the levels of antibodies in individuals in the other psychiatric groups did not differ significantly from the levels measured in the control population (multi-episode schizophrenia, t = 0.04, p = 0.967; bipolar disorder not selected for mania, t = -0.25, p = 0.803; recent onset psychosis, t = -1.47, p = 0.143). In all of these analyses we adjusted for age, race, gender, and maternal education. There were significantly increased odds of increased levels of NR2 antibodies (≥ 90th percentile of the controls) in the mania group at the time of admission (OR = 2.78, 95% confidence interval: 1.26–6.14, p = 0.012) but not in the mania group at other time points or in the other psychiatric groups. Among all of the psychiatric participants, the levels of antibodies at evaluation did not differ significantly based on whether the individual was treated with lithium, anticonvulsant, antidepressant, or antipsychotic medication (all p > 0.30).

We also examined whether individuals with mania had increased levels of antibodies to other human antigens. The levels of antibodies to double-stranded DNA and tissue transglutaminase in the individuals with mania did not differ from those measured in controls (p > 0.05 adjusted for age, gender, and race).

**Discussion**

Our study documents that individuals hospitalized for mania have increased levels of antibodies to the NR2 peptide fragment of the NMDA receptor. It is of note that the antibody levels were not significantly elevated as compared to controls at the time of the six-month follow-up evaluation. The reasons for the decrease in antibody levels after initial hospitalization for mania are not known with certainty but may be related to treatment or to the course of the underlying disorder. Significant elevations in NR2 antibodies were not found in the other psychiatric groups, including individuals with bipolar disorder who were not selected for mania as well as individuals with the recent onset of psychosis or multi-episode schizophrenia.

![Fig. 1](image-url)

**Fig. 1.** Levels of antibodies associated with the indicated study groups as described in the text. The groups tested and the number of individuals in each group other than mania were as follows: Control, individuals without a psychiatric disorder, n = 153; Multi episode Scz, individuals with multi-episode schizophrenia, n = 257; Rec ons psychosis, individuals with recent-onset psychosis, n = 49; bipolar disorder, individuals with bipolar disorder not selected for mania, n = 61. The samples from 60 individuals with mania were designated as follows: Admission, samples obtained on admission to the hospital, n = 44; Baseline, samples obtained later in the hospital stay on enrollment in the study, a mean of 4.0 days (SD = 2.3) following admission, n = 60; Follow-up, samples obtained at follow-up, a mean of 191.7 days (SD = 13.3) following enrollment, n = 39. *p = 0.01; **p = 0.003, compared to controls, adjusted for age, gender, race, and maternal education.
Our results highlight the extent to which NMDA receptor dysfunction may be contributing to the pathophysiology of mania. NMDA receptors are glutamate receptors with roles in synaptic transmission and plasticity. Several lines of evidence suggest that glutamatergic system dysfunction, particularly the NMDA receptor complex, may play a role in the pathophysiology of bipolar disorder (24, 25). These include studies of post-mortem brain tissue indicating abnormalities in components of the glutamatergic system (26) as well as genetic studies indicating an increased risk of bipolar disorder in individuals with variants in the genes encoding glutamate receptor complexes (27). In addition, NMDA receptors are implicated in the function of the PKC signaling pathway; dysregulation of this signaling cascade has been associated with the biological substrate of mania (8, 9). The individuals with mania in our study did not have increased levels of antibodies to two additional human antigens, double-stranded DNA and tissue transglutaminase. Since antibodies to these antigens are often elevated in autoimmune disorders, this finding indicated that the increased levels of antibodies to NR2 cannot be ascribed to a generalized increase in autoimmune reactivity. The mechanisms associated with the generation of antibodies to the NR2 receptor are not known with certainty but may be related to exposure to infectious agents and/or immune activation in genetically susceptible individuals. These mechanisms should be the focus of additional investigations in humans and experimental animals.

There is a single case report of antibodies to the NR2 subunit in the blood and CSF of a patient with acute mania (16). It is of note that the individual described in this report developed signs of encephalitis such as movement disorder, reduced level of responsiveness, and autonomic dysfunction after admission for acute mania, so the presentation in this report is different from that of the individuals we studied, who did not have clinical signs of encephalitis.

Strengths of our study included the relatively large total sample size and the number of comparison groups, and the fact that all of the participants were drawn from the same geographic area and, in the case of the patients, the same psychiatric health care system. In addition, the fact that our mania group included different subtypes, including schizoaffective cases. Also, while we were able to study the mania group over time, the individuals in the other psychiatric groups and controls were only evaluated at one point in time. Additional longitudinal studies should be performed to determine if individuals with other psychiatric disorders have elevated levels of antibodies to NR2 at some time point during the course of their disease. Also, since we did not perform lumbar punctures, we could not measure the levels of NR2 antibodies within the CNS. However, the fact that the levels we measured in the serum decreased after the acute mania episode suggests that the levels of these antibodies measured in the periphery correlate with events occurring within the CNS. Additional studies should be performed to define the pathophysiological effects of antibodies to the NMDA receptor within the CNS of individuals with mania. It would also be informative to obtain information about other symptoms that may be related, such as neurological symptoms.

It is of note that many of the individuals in our mania group improved clinically in the six-month follow-up period after their initial clinical evaluation during hospital admission and the institution of lithium and other medications commonly used for the treatment of mania. However, recent studies have documented the need for improved therapies for mania (28). It is of note that some individuals with anti-NMDA-associated encephalitis have been reported to show clinical improvement following treatment with immune modulating agents (17, 29). The possible role of immune modulatory therapy in individuals with mania who have increased levels of antibodies to NR2 should be the subject of future clinical trials. The successful conclusion of such trials might lead to new modalities for the treatment of mania in some individuals.

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Disclosures
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