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A mouse model of seizures in anti-N-methyl D-aspartate receptor encephalitis

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Summary

Objective: Seizures develop in 80% of patients with anti-N-methyl D-aspartate receptor (NMDAR) encephalitis, and these represent a major cause of morbidity and mortality. Anti-NMDAR antibodies have been linked to the memory loss in encephalitis; however, their role in seizures has not been established. We determined whether anti-NMDAR antibodies from autoimmune encephalitis patients are pathogenic in causing seizures.

Methods: We performed continuous intracerebroventricular infusion of cerebrospinal fluid (CSF) or purified immunoglobulin (IgG) from the CSF of patients with anti-NMDAR encephalitis or polyclonal rabbit anti-NMDAR-IgG, in male C57BL/6 mice. Seizure status during a 2-week treatment was assessed with video electroencephalography. We assessed memory, anxiety-related behavior, and motor function at the end of treatment and assessed the extent of neuronal damage and gliosis in the CA1 region of hippocampus. We also performed whole-cell patch recordings from the CA1 pyramidal neurons in hippocampal slices of mice with seizures.

Results: Prolonged exposure to rabbit anti-NMDAR-IgG, patient CSF or human IgG purified from the CSF of encephalitis patients induced seizures in 33 of 36 mice. The median number of seizures recorded in 2 weeks was 13, 39 and 35 per mouse in these groups, respectively. We observed only 18 brief nonconvulsive seizures in 11 out of 29 control mice (median seizure count

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Disclosure
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of 0) infused with vehicle (n=4), normal CSF obtained from patients with noninflammatory CNS conditions (n=12), polyclonal rabbit IgG (n=7), albumin (n=3) and normal human IgG (n=3). We did not observe memory deficits, anxiety-related behavior, or motor impairment measured at 2 weeks in animals treated with CSF from affected patients or rabbit IgG. Furthermore, there was no evidence of hippocampal cell loss or astrocyte proliferation in the same mice.

**Significance:** Our findings indicate that autoantibodies can induce seizures in anti-NMDAR encephalitis and offer a model for testing novel therapies for refractory autoimmune seizures.

**Keywords**

autoimmune seizures; animal model; anti-NMDAR antibodies; autoantibodies; refractory seizures

1. **Introduction**

Anti-NMDAR encephalitis is a prevalent autoimmune encephalopathy that surpassed the incidence of viral encephalitis in the California encephalitis project. The disease is characterized by confusion, psychosis, dysautonomia, as well as movement disorders, and is often associated with persistent seizures that require treatment with pharmacologically-induced coma. New-onset seizures have been diagnosed in 78–86% of patients with anti-NMDAR encephalitis and have been reported in all stages of the illness. The spectrum of clinical presentation of seizures includes purely electrographic seizures, generalized convulsions, and focal seizures with loss of awareness. Particularly, severe treatment-resistant seizures were initially reported in 6% of patients; however, a growing number of reports suggests that this number may have been grossly underestimated. Thus, in the recent multicenter study of patients with anti-NMDAR encephalitis, 42% required intensive care unit settings and convulsive or non-convulsive status epilepticus was present in 45% of these patients. Moreover, status epilepticus was deemed refractory to anticonvulsants in two thirds of patients.

While overall clinical improvement in nonparaneoplastic anti-NMDAR encephalitis occurs in less than 50% of patients following first-line immunotherapy, seizures appear to respond well to conventional anticonvulsants in the majority of patients. The presence of NMDAR antibodies associates with seizures, but their pathogenic role has not been established. Infusion of anti-NMDAR antibodies in mice did not cause seizures unless their seizure threshold had been reduced by the chemical convulsant, pentylenetetrazol.

The reason for the unsuccessful attempts to reveal spontaneous seizures could be a short duration of treatment with antibodies, or that the seizures might have been exclusively electrographic and present without behavioral correlates. Therefore, in previous studies of mice infused with NMDAR-IgG-positive patients’ CSF, nonconvulsive seizures may not have been detected because simultaneous electrographic monitoring was not performed. The lack of an animal model of autoimmune epilepsy limits our understanding of the mechanisms of seizure production and hampers the development of new treatments for these patients.

Recent in vitro studies showed that patients’ antibodies caused reversible titer-dependent internalization of NMDAR synaptic clusters and decreased cell-surface receptor density.
Furthermore, antibodies decreased synaptic NMDAR-mediated currents in cultured hippocampal neurons. In mouse hippocampal slices exposed to CSF positive for anti-NMDAR antibodies, impaired long-term potentiation (LTP) was observed. In addition, short-term intracerebroventricular (icv) administration of patients’ CSF to rats led to decreased excitatory postsynaptic potentials in hippocampal granule cells that was accompanied by the impairment of memory and learning. Thus, the loss of NMDAR-mediated synaptic function, observed most prominently in the hippocampus in anti-NMDAR encephalitis, could explain the cognitive impairment; however, the question remains whether these effects are accompanied by spontaneous seizures.

In the present study, we assessed the role of anti-NMDAR antibodies in the development of autoimmune seizures in mice by administering continuously via icv route rabbit anti-NMDAR antibodies or CSF from affected patients and measuring seizure activity by EEG. We also examined the behavioral phenotype of mice with antibody-induced seizures and assessed hippocampal pathology. The data in this study provide evidence that autoantibodies in NMDAR encephalitis are pathogenic for seizures. The mouse model of seizures in anti-NMDAR encephalitis characterized in these experiments can be used to assess novel therapies for this devastating acute-onset epilepsy, as well as provide a means to study other still cryptogenic epilepsies, such as new-onset refractory status epilepticus.

2. Methods

We used male C57BL/6 mice that were 8–10 weeks old at the time of electrode implantation. Experimental polyclonal antibodies against the N-terminal domain of human GluN1 protein and experimental CSF specimens (provided by S.J.P.) were pooled from clinical residuals from eight patients with anti-NMDAR encephalitis and high CSF antibody titer (Supplemental Fig. 1 in S1) and one patient of O.T. with anti-NMDAR encephalitis and seizures. Control CSF pooled from three patients with noninflammatory diseases of the central nervous system had no detectable anti-NMDAR antibodies by both tissue- and cell-based indirect immunofluorescence. Purified patient antibodies in the IgG fraction of CSF (experimental) and normal serum human IgG (control) were purified from clinical residuals of patients with anti-NMDAR encephalitis and healthy volunteers, respectively (provided by S.J.P.). See supplementary S1 for detailed descriptions of animals, drugs, and CSF specimens.

2.1 Stereotaxic surgery and osmotic minipump insertion

Mice were stereotactically implanted with injector guide cannula into the lateral ventricle, a head mount for 2 EEG/1 EMG channels (Pinnacle Technology Inc., Lawrence, KS) and two cortical screw EEG electrodes placed to record signals from the parietal cortex overlying hippocampus (EEG 1) and ipsilateral frontal cortex (EEG 2). After a 7-day recovery period, mice were implanted with osmotic minipumps filled with antibody solution, CSF, or vehicle; the pumps were connected to the guide cannula. Animals underwent continuous icv infusion of patients’ CSF or rabbit anti-NMDA IgG or PBS, polyclonal rabbit IgG or normal CSF (n=4–9) for 2 weeks at a flow rate of 0.25 µl/h (Fig. 1); the latter three groups were combined into a single control group for analysis (surgical details in S1). A similar
experimental paradigm was used to infuse purified patient anti-NMDAR antibodies in the IgG fraction of normal CSF, human albumin, and normal human serum IgG (n=3 in each group).

2.2 EEG acquisition and analysis of seizure patterns

The EEG system (Pinnacle 8206, Pinnacle Technology) was comprised of a preamplifier unit connected by a tether to a conditioning/acquisition system. Signals were sampled at 400 Hz (preamplifier gain at 100X, total gain 5,000X, high pass EEG channel filter: 0.5 Hz, low pass EEG filter: 50 Hz), digitized using a 14-bit analog to digital converter and routed to a PC (personal computer).

EEG signals were categorized as normal or seizures (greater than 5 sec of sustained, rhythmic high amplitude activity in both EEG leads)\(^{26}\). Events separated by >10 sec of baseline activity were defined as distinct events. Seizure behavior was assessed using a modified Racine scale \(^{27}\) during seizure and 10 sec thereafter; events attributed to extraneous motor movements generated by normal mouse behavior were ignored (details in S1).

2.3 Behavioral assays

Upon completion of 2-week EEG recording, animals underwent assessment of memory, anxiety-related behavior, and motor function in novel object recognition, open field and accelerated rotarod tests, respectively (Fig. 1; details in S1).

2.4 Histopathology

Upon completion of behavioral experiments, animals were sacrificed and brains were processed for immunochemistry for GFAP (astrocytes) and fluoro-Jade C (damaged neurons) as previously described \(^{28}\). Histologic quantification of these cells in hippocampi was performed using ImageJ (NIH).

To compare the gliosis in CSF-perfused mice with an epilepsy model known to result in gliosis, sections of brain from mice with pilocarpine-induced status epilepticus were processed as described in S1.

2.5 Whole cell patch recordings in hippocampal slices

Hippocampal slice preparations and recordings paradigms were performed as described \(^{29}\) (see S1 for details).

2.6 Statistical analysis

Data for median seizure counts and total time in seizures were not normally distributed therefore, the Kruskal-Wallis and Dunn’s tests with selected comparisons (or Mann-Whitney tests) were used (Graph Pad Prizm 7, La Jolla, CA). Means (latency to the first seizure, the daily seizure burden scores, cognitive and locomotor scores) were compared using analysis of variance (ANOVA). Diurnal seizure count fluctuations and total seizure counts during the light and dark cycles were compared by repeated measure ANOVA and t-test, respectively. GFAP-positive cell counts were compared between vehicle and pilocarpine-treated mice using unpaired t-test and among the combined control, CSF- and rabbit IgG-treated mice.
using ANOVA. Data for the whole cell patch recordings in hippocampal slices were expressed as the mean ± SEM and analyzed using paired t-test (excitability) or Student’s unpaired t-test (all other data). Differences with p < 0.05 were considered statistically significant. The median seizure duration, the inter-seizure intervals (ISI) between successive seizures and median ISI in mice treated with human CSF were calculated with Microsoft Excel; a distribution function of number of seizures and intervals were generated and plotted with Origin (OriginLab, Northampton, MA). The ISI was compared to ISI of 190 min (expected if seizures occurred evenly throughout the 2-week recording period) with one sample t-test. The diurnal seizure count variations were assessed with repeated measure ANOVA while the total seizure counts during the light and dark cycles were compared using t-test. The sample size was estimated based on the previously published studies using comparable experimental protocols. EEG tracings generated at UNMC are available from the corresponding author on request.

3. Results

3.1 CSF from patients with anti-NMDAR encephalitis induces seizures in mice

We infused purified rabbit anti-NMDAR-IgG (0.2 µg/µl) continuously into the lateral ventricles of mice for 14 days and found that prolonged exposure to antibodies induced seizures in 10 out 11 animals (Fig. 2A; p < 0.001 Kruskal-Wallis test; p = 0.006, Dunns’ multiple comparisons tests vs. combined control). Using the same experimental conditions, we performed a passive transfer of CSF from a single patient or high titer pooled CSF of patients with anti-NMDAR encephalitis. Consistent with the effects of anti-NMDAR-IgGs, CSF from affected patients induced seizures in 16 out of 17 mice (Fig. 2A; p < 0.0001, Dunns’ multiple comparison test). The variability in seizure counts in mice is expected from the clinical seizure phenotype in patients who demonstrate wide variability in seizure burden. Data from the corresponding control groups, which included vehicle (n=4), normal CSF obtained from patients with noninflammatory CSF conditions (n=9), and polyclonal rabbit IgG (n=7), were combined for the analysis. There were total of 12 brief nonconvulsive seizures in 5 out of 20 control mice. Specifically, 2, 4, and 6 seizures were recorded in mice treated with vehicle, rabbit IgG, or control CSF, respectively (Fig. 2 A-C). There were no significant differences in the number of seizures among the three control groups (p = 0.54, Kruskal-Wallis test).

The median number of seizures recorded during the 2-week period in mice treated with rabbit anti-NMDAR IgG and patient CSF was 13 (interquartile range, IQR 8–33) and 39 (IQR 10–157), respectively. The median number of seizures in the combined control group was 0 (IQR 0–1). As expected, the median seizure counts were significantly higher in mice treated with rabbit anti-NMDAR IgG and patient CSF compared to the control (p = 0.0006 and p < 0.0001 vs. control, respectively; Dunn’s tests). The median total time in seizures over the 2-week period in the combined control, rabbit anti-NMDAR IgG, and patient CSF-treated groups (median and IQR) were 0 (0–0.24), 29.8 (10.2–47.9), and 75.6 (13.3–358) min, respectively (Fig. 2B). Mice treated with NMDAR antibody and patients’ CSF spend more time in seizures compared to the control mice (p < 0.0001, Kruskal-Wallis test; p = 0.0005, and p < 0.0001, Dunn’s tests). The latencies to first seizure (mean ± SEM) in mice

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infused with anti-NMDAR IgG solution and patient CSF were not different from those in control mice (66.4 ± 28.1; 50.4 ± 17.3, and 46 ± 20.1 h, respectively (p = 0.78; ANOVA; data not shown).

Ninety-three percent of seizures recorded in mice treated with antibodies or patient CSF were exclusively electrographic and 2% of seizures were accompanied by only minimal behavioral changes corresponding to the modified Racine’s scores 1–3 \(^27\) (Fig. 2C and Supplemental video 1). More severe seizures characterized by isolated myoclonic jerks and sustained clonic activity (scores 4 and 5, respectively) occurred in a few animals and constituted 5% of all seizures in these mice. The few seizures in the combined control infusion group were all nonconvulsive (Fig. 2C). The median seizure duration in mice treated with CSF from patients with anti-NMDAR encephalitis was 62.4 sec (IQR 8.97–434) sec (Fig. 2D).

Consistent with the effects of the whole CSF from affected patients, patient IgG purified from anti-NMDAR antibody-containing CSF also induced seizures in mice (median = 35 in 2 weeks; IQR 0–139; \(p = 0.02\); Mann-Whitney test vs. combined control; Fig. 2E). Seizures occurred in 6 out of 8 mice, and the median time spent in seizures over the 2-week period was 66.7 (2.7–292.2) min (data not shown). There were total of 6 nonconvulsive seizures in 4 out of 9 animals from the combined control group (median 0, IQR 0–1.5) comprised of mice treated with normal human CSF (n=3), human albumin (n=3), and normal human serum IgG (n=3). There were no significant differences between the number of seizures in the latter subgroups (\(p = 0.89\), Kruskal-Wallis test). The total time spent in seizures in the patient IgG-treated group was significantly longer than that in the control (\(p = 0.02\), Mann-Whitney test). In parallel to seizure presentation in mice treated with whole CSF from affected patients, 95% of seizures in mice were exclusively electrographic (data not shown). The latencies to first seizure (mean ± SEM) in mice infused with patient anti-NMDAR containing IgG were not different from those in control mice (118.6 ± 47.1 and 36.9 ± 26.7 h, respectively (\(p = 0.15\); t-test, data not shown).

The majority of seizures were characterized by high amplitude rhythmic spikes that occurred at relatively constant rates or at irregular intervals (Fig. 3A and Supplemental video 1). In the former pattern, the spikes occurring at relatively constant intervals had larger amplitude and duration compared to the latter which was characterized by spikes of varied amplitude occurring at irregular intervals. The corresponding behavior varied from sleeping or normal exploratory activity or freezing and myoclonic jerks (Fig. 2C, Supplemental videos 1 and 2). A less common seizure pattern was characterized by high amplitude fast rhythmic activity that fluctuated in amplitude in a spindle-like fashion (Fig. 3B). Both patterns could occur in the same animals. The seizures observed in control mice were largely characterized by rhythmic spikes occurring with constant frequency.

We also performed whole cell patch recordings from CA1 pyramidal neurons in hippocampal slices prepared from control and anti-NMDAR-positive CSF animals. The resting membrane potential, amplitude, and frequency of spontaneous EPSCs and IPSCs were similar in the two treatment groups. The input resistance and response to depolarizing
current injection were reduced in cells from the antibody-infused animals (p = 0.02 and p = 0.002, respectively, unpaired and paired t-tests, respectively; Supplemental Fig. 2 in S1).

3.2 Antibody-induced seizure timing does not demonstrate daily or diurnal fluctuations

To determine the temporal distribution of seizure burden (i.e. total number of seizures) during the continuous exposure to patients’ CSF, the temporal fluctuation of seizure occurrence was assessed in mice with high (> 39) and low (≤ 39, split was based on the median seizure count) seizure burden over the 2-week period (8 mice in each group; Fig. 4A). As expected, the mean daily seizure scores were significantly higher in mice with high seizure burden compared to those with low seizure burden (group effect: p = 0.047; two-way ANOVA). The seizure scores in the high seizure burden group were higher on days 11–15 but not on days 1–5 and 6–10 (p = 0.02; p = 0.32, and p = 0.99, respectively; Sidak multiple comparison tests). The triaging of mice into groups with low or high seizure burden over the first week, and the seizure score on days 11–15, can together be applied to determine the response to treatment for seizures in future interventional studies.

In order to determine if seizure timing exhibits a diurnal pattern, seizure burden within the light and dark cycles was examined by pooling all seizures recorded in 2 weeks in 8 CSF-administered mice with high seizure density in successive 2h intervals (Fig. 4C). The data indicate that only minimal diurnal variations occurred in the seizure count (F$_{3.1; 21.5}$ = 1.17, p = 0.34; repeated measures ANOVA), and the total median seizure counts during the light and dark cycles were not different (p = 0.72, t-test; Fig. 4D).

To determine whether seizures tended to occur in clusters (cf. 30), we examined the inter-seizure intervals of 1,694 spontaneous seizures pooled from 16 mice perfused with patients’ CSF for two weeks. The inter-seizure interval histogram was approximately normal with median interval of 9.5 min (IQR 1.7–52.8 min) (Fig. 4D). Because evenly-spaced seizures would have occurred every 190 min, this analysis provides strong evidence for seizure clustering in this mouse model (p < .0001 for measured ISI compared to expected ISI of 190 min, one sample t-test). Indeed, fewer than 4% of all seizures had an inter-seizure interval >190 min, whereas >20% of seizures occurred with ISI of <1 min.

3.3. Anti-NMDAR antibodies did not alter behavior in mice

In the novel object test, the recognition scores (mean ± SEM) in the combined control, anti-NMDAR IgG, and patient CSF-treated mice were 0.60 ± 0.05, 0.55 ± 0.06, and 0.51± 0.05, respectively (Fig. 5A). Seven out of 20, 3 out of 11, and 5 out 17 mice were excluded from the above groups, respectively because these mice failed to spend more than 4.5 sec in total exploration. The recognition scores in the control group were similar to those previously reported by other authors 31 and recorded in our laboratory. However, we note that although the control group of mice tended to exhibit a bias in favor of the novel stimulus, this trend did not reach statistical significance (p = 0.09; one sample t-test). The antibody- and patient CSF-treated mice spent equal time exploring the novel and familiar objects (p = 0.46 and p = 0.9, respectively; one-sample t-tests comparing with the recognition score of 0.5); however, there were no differences in the novel object recognition scores among three treatment groups (F$_{2,30}$ = 0.92; p = 0.41; ANOVA). These findings fail to provide evidence that non-
spatial learning and memory are affected in a novel object paradigm shortly after a 2-week infusion of antibodies.

When exposed to the open field, the total distance traversed by mice (mean ± SEM) in the combined control, IgG and patient-CSF-treated groups were 53.1 ± 4.8, 59.7 ± 2.8, and 61.0 ± 4.4 m (Fig. 5B). Furthermore, the proportion of time spent in the inner zone of the arena in the same groups were 16.9 ± 2.3, 14.2 ± 1.4, and 12.8 ± 1.7 percent, respectively (Fig. 5C). The locomotor and anxiety scores in the control group in our study were comparable to those previously reported in similar experimental paradigms. Consistent with the findings by others, antibodies did not affect the locomotor activity and emotion-related behavior in mice (F$_{2,44} = 1$, p = 0.36; and F$_{2,44} = 1$, p = 0.31, respectively; ANOVA). One control mouse treated with normal rabbit IgG was excluded from calculations because of data loss caused by acquisition software malfunction.

The mean latencies to fall from the rotarod (mean ± SEM) in the three groups were 30.2 ± 3.7, 22.8 ± 3.0, and 22.5 ± 1.7 sec (Fig. 5D). The differences among the groups did not reach statistical significance (F$_{2,45}$, 2.18, p = 0.12; ANOVA).

3.4 Antibody treatment induced mild glial activation but not proliferation or neuronal death in the hippocampus

Sections of CA1 region of the hippocampus stained for GFAP immunoreactivity demonstrated a characteristic pattern of immunostaining and the glial cells had a typical morphological appearance (Fig. 6). The gliotic cells in anti-NMDAR patient CSF-treated mice (Fig. 6 C,D) and pilocarpine-treated mice with status epilepticus (used as a positive control, Fig. 6 E,F) were identified by the presence of thick tortuous processes and more intense staining as compared to the GFAP-positive cells with thin lightly-stained processes in naive mice (Fig. 6A,B). Fluorojade C staining for degenerating neurons did not demonstrate any signs of neurodegeneration in the CA1 region of hippocampus in mice with antibody-induced or pilocarpine-induced seizures (data not shown). The number of GFAP-positive cells per mm$^2$ in mice treated with control CSF, rabbit anti-NMDAR IgG, or patient CSF was similar among the infused groups (Supplemental Fig. 3 in S1). There was no evidence of astrocyte proliferation in mice-treated with purified anti-NMDAR IgG or patient CSF (F$_{2,35}$ = 2.51; p = 0.03; ANOVA; p = 0.51 and p = 0.1, anti-NMDAR IgG and patient CSF groups vs. combined control group, respectively; Dunnett’s multiple comparison tests). Although visually the astrocyte staining appeared darker in mice infused with patient CSF (Fig 6), variability in the background intensity precluded quantification of this impression.

4. Discussion

This study demonstrates that antibodies to NMDAR receptors can cause spontaneously occurring electrographic seizures. Similar to the patients with anti-NMDAR encephalitis, 80–89% of whom develop seizures, 75–93% of antibody-infused mice in the present study developed seizures. By comparison, only a few seizures were observed in control mice. Mice receiving CSF with high seizure burden could be enrolled into trials that test the response to novel anticonvulsant treatments and daily seizure score could be used to follow these effects.
Since the initial description of anti-NMDAR encephalitis in a patient with sudden reversible psychosis, major efforts have been exerted to establish the role of autoantibodies in the pathophysiology of the encephalitis phenotype, including seizures. Given that spontaneous or treatment-induced removal of the antibodies in patients with anti-NMDAR encephalitis leads to improvement in confusion, psychosis, and seizures, the antibodies were assumed to be responsible for the development of these symptoms. Whereas seizures in anti-NMDAR encephalitis rarely persist into the late convalescent period, they are present in the majority of patients during the acute phase and fail to respond to conventional antiepileptic treatments in 15–45% of patients. The anti-NMDAR antibodies found in some patients with encephalitis bind to an epitope spanning amino acid residues 144–156 in the N-terminal domain of the GluN1 subunit, affect synaptic trafficking of the adjacent GluN2A and 2B subunits, and cause internalization of NMDAR clusters.

While the antibody-associated human memory deficits were reproduced in previous studies in rodents, spontaneous seizures were not observed in mice following a single infusion of anti-NMDAR encephalitis CSF. Overt seizures were also not reported in a study with continuous icv infusion of CSF from patients with anti-NMDAR encephalitis; however, simultaneous EEG recording was not performed. The majority of seizures in the present study were accompanied by subtle or no motor signs; this is consistent with clinical presentation in patients with encephalitis, many of whom develop subclinical seizures and require EEG monitoring for seizure diagnosis. Exclusively electrographic seizures with spike and wave pattern are not uncommon in rodents and have been previously reported in rodent models of limbic and kainate-induced epilepsy as well as in ischemic-hypoxic seizures. Minimal behavioral changes (e.g., freezing) or lack of motor signs occur because seizures likely occur in discrete cortical regions and do not propagate to the areas responsible for motor control. In the present study, seizures caused by commercial polyclonal NMDAR IgG were similar to those produced by encephalitis-patient CSF and the purified IgG fraction from patient CSF suggesting that seizures were directly caused by the effects of antibodies rather than being induced by other constituents of patients CSF (e.g., proinflammatory cytokines) that could also promote the development of seizures. Furthermore, these findings indicate that exposure to antibodies must be sustained to induce seizures and that detection of seizures requires electrographic monitoring.

The pathophysiology of seizures as a consequence of NMDAR dysfunction appears to be more complex than their cellular dysfunction would suggest. A genetically-induced ablation of the GluN1 subunit of NMDARs in GABAergic corticolimbic neurons of mice resulted in memory loss and anxiety, a phenotype similar to that observed in rodents infused with CSF from encephalitis patients, suggesting that seizure behavior might also be similarly induced by cell-specific NMDAR hypofunction in encephalitis. Recent studies showing markedly enhanced excitability of the motor cortex in rodents treated with patients’ anti-NMDAR antibodies provide an early insight into the mechanisms of seizures related to NMDAR dysfunction. Specifically, these results supported a direct role of anti-NMDAR antibodies from encephalitis patients in altering glutamatergic neurotransmission. Furthermore, excessive glutamate release in the hippocampi of rats was reported following a single intracerebral administration of anti-NMDAR encephalitis CSF, supporting the concept that patients’ antibodies may contribute to the removal of inhibitory...
tone and excessive pyramidal cell firing. While extracellular glutamate was not measured in the present study, continuous infusion of antibody-positive CSF could induce heightened glutamate release in the hippocampus. Thus, seizures may develop in the setting of elevated extracellular glutamate if the impact of antibody-induced NMDAR hypofunction preferentially affects inhibitory interneurons. However, no functional evidence of interneuronal NMDAR hypofunction has been reported (see also Supplemental Fig. 2). The decrease in inhibitory synapse density onto excitatory hippocampal neurons was found in culture after acute exposure to patients’ CSF. The prolonged exposure to antibodies may lead to similar adaptations at the circuit level. Thus, the psychosis manifesting in patients with anti-NMDAR encephalitis might be caused by the NMDAR hypofunction on inhibitory interneurons and the resultant disinhibition of cortical glutamategic and dopaminergic projections.

While the locus of seizure onset cannot be precisely determined without EEG recordings from a panel of depth and surface electrodes, the seizures detected over the parietal cortex in the present study could emanate from the hippocampus, immediately inferior to the parietal cortex. Hippocampi of mice that completed a 2-week icv infusion of patients’ CSF had the highest density of the brain-bound NMDAR IgGs and the most robust decrease of NMDAR clusters. Paradoxically, in recordings from hippocampal slices isolated from rats injected intracerebrally with anti-NMDAR encephalitis CSF, the action potential threshold of hippocampal pyramidal neurons was higher than that in controls treated with normal CSF. Consistent with that, we found that excitability and membrane resistance of granule cells in slices of mice treated with patient CSF were reduced compared to those in control mice (Supplemental Fig. 2). Further studies are needed to determine if the lowered excitability is homeostatically responsive to the recurrent seizures or is directly mediated by the effects of patients’ autoantibodies.

In mouse hippocampal slices, CSF positive for anti-NMDAR antibodies impaired LTP. In addition, both short-term and prolonged administration of patients’ CSF to rodents led to the impairment of memory and learning. In the present study, mice treated with commercial antibodies or patient CSF failed to discriminate between the novel and familiar objects based on one sample t-tests, which is consistent with memory impairment and encephalitis phenotype. However, since a strong preference for the novel object was not observed in control mice, the present assay was likely not sensitive enough to assess non-spatial memory in this model. Another possibility for lack of the expected response in the control animals was an iatrogenic consequence of extensive cranial surgery. The lack of memory impairment was consistent with the previous findings and could have been due to testing prior to the peak worsening. Alternatively, the novel object paradigm applied in our study was not sensitive enough to reveal the differences between the three treatment groups in the setting of additional cerebral instrumentation. Additional tests to demonstrate antibody-induced alteration of memory in mice may need to be applied to better characterize the impairment in multiple domains of memory function. The lack of anxiety-related behavior and unaltered locomotor activity in antibody-treated mice in the present study agreed with previous reports. Taken collectively, these findings suggest that a single animal model may not be suitable to reproduce various clinical symptoms of anti-NMDAR encephalitis (e.g., memory loss, seizures, and dyskinesia).
The neuropathology of anti-NMDAR encephalitis in autopsy specimens of affected patients and in brain tissue sections of rodents treated with patients’ CSF involves deposition of IgG in the hippocampus and cortex without evidence of destruction of the nerve cells or synapses. The absence of evidence for neuronal death in the hippocampi of mice in this study is therefore consistent with the previous studies. Also consistent with previous reports of astrocytic hypertrophy in seizures, we observed morphological changes in hippocampal astrocytes of mice infused with purified IgGs or patients’ CSF; however, we found no evidence of astrocytic proliferation in these animals. Such a dissociation between the qualitative and quantitative changes in astrocytes following seizures was previously reported in cortical neurons of mice with kainate-induced status epilepticus.

A limitation of the present study includes the lack of clinical information on all but one patient from whom the CSF was derived, thus restricting more complete translation of findings to the bedside. Given the very high titer of anti-NMDAR antibodies in the CSF from individual patients and that of the final pool of purified IgG (1:1024), the specimens were likely obtained prior to the initiation of treatment. These patients were likely suffering from severe encephalitis; however, it is unclear whether they had developed overt seizures. Another limitation stems from the constraints of the EEG recording in mice, which is challenging to fully distinguish between behavior-related EEG artifacts and rhythmic seizure patterns. Thus, the number of seizures and the severity of epileptic phenotype in mice could be underestimated. Furthermore, rhythmic patterns appearing only in a single EEG lead were excluded; thus, focal seizures were uncounted. Future recordings from depth electrodes in targeted brain regions or the use of a wireless EEG system may reduce movement-related artifacts. The CSF from patients from non-inflammatory CNS conditions used as a control in our study was previously validated in other animal models of autoimmune encephalitis and related in vitro studies. An additional control group of mice with the CSF from affected patients but having anti-GluN1 antibodies removed by means of immunodepletion can be used in the future experiments.

In conclusion, this study provides the first demonstration of the pathogenicity of neuronal antibodies for seizures in autoimmune encephalitis. The mouse model developed herein can be applied to test novel pharmacological treatments for refractory autoimmune seizures. This model can also be used to study the etiology of seizures in autoimmune epilepsy, examining CSF itself in comparison with other potentially pathogenic components. Furthermore, this study provides a foundation for future in-depth examination of the effects of antibodies on extended hippocampal circuitry that could elucidate new targets for therapeutic neurostimulation in autoimmune epilepsy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References


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Key Points

- CSF or purified IgG from patients with anti-NMDAR encephalitis, as well as polyclonal rabbit anti-NMDAR IgG antibodies, induced seizures in mice.
- Antibody-induced seizure timing did not demonstrate daily or diurnal fluctuations.
- Mice exposed to patients’ CSF or anti-NMDAR IgG antibodies did not develop memory deficits nor did they show anxiety-related behavior or motor impairment.
- Excitability and membrane resistance of CA1 pyramidal neurons in mice hippocampal slices treated with patient CSF were reduced compared to those in control mice.
Figure 1.
Experimental protocol to assess the role of antibodies in seizure responses during the infusion, followed by behavioral and neurophysiological analysis.
Figure 2.
Mice treated with CSF from patients with anti-NMDAR encephalitis or rabbit anti-NMDAR antibody develop seizures. (A) Total seizure counts and (B) total time in seizures during video EEG monitoring of mice treated for 2 weeks with continuous icv infusion of anti-NMDAR antibody solution (open triangles) or human CSF (open and closed circles). Green, red, and blue closed circles represent PBS-, normal rabbit IgG, and normal CSF-treated mice, respectively. Horizontal bars indicate median values for combined control group (n=20), rabbit anti-NMDAR IgG (n=11), and patients’ CSF (n=17). (C) Behavioral...
expression of antibody-induced seizures. The number of seizures observed for each Racine score is indicated. (D) The log of the seizure duration in mice treated with patients’ CSF (n=17) histogram has a normal distribution ($R^2 = 0.995$) with the peak at 1.77 corresponding to a median seizure duration of 58.9 sec. (E) Total seizure counts in mice treated with patient anti-NMDAR antibodies in the IgG fraction purified from the pooled CSF (final titer 1:1024), normal human CSF, human albumin and normal human serum IgG (blue, purple and orange closed circles, respectively). $p = 0.02$. *, $p < 0.05$; **; $p < 0.01$; ***; $p < 0.001$; ****, $p < 0.0001$ vs. control, Dunns’ selected comparison tests (A, B) or Mann-Whitney test (E).
Figure 3.
Tracings of the nonconvulsive seizures in mice treated with continuous icv infusion of CSF from patients with anti-NMDAR encephalitis. (A) Seizure initiation with a single spike followed by irregular spike pattern. (B) Fast rhythmic activity that fluctuates in amplitude.
Figure 4.
Seizure burden in mice treated with CSF from patients with anti-NMDAR encephalitis. (A) Time-course of daily seizure counts (mean ± SEM) in mice that exhibited >39 or ≤39 seizures in 2 weeks (high or low seizure burden, respectively). (B) The log of the inter-seizure interval histogram has a normal distribution. A total of 1,694 seizure intervals were pooled from 16 mice perfused with patients’ CSF and recorded for 2 weeks. The log histogram is reasonably well fit by a Gaussian model ($R^2 = 0.944$) with peak at 0.982 (= 9.6 min) and log SD = 1.11. (C) Seizure counts in CSF-treated mice with high seizure burden (mean ± SEM) had minimal diurnal fluctuations. Daily seizures recorded in 2 weeks in 8 CSF-perfused mice with high seizure density were pooled in successive 2h intervals. (D) Daily seizure counts during the light and dark cycles in CSF-treated mice with high seizure burden were not different ($p = 0.72$, t-test).
Figure 5.
Effects of antibodies on the behavioral phenotype. Two-week treatment with rabbit anti-NMDAR IgG or CSF from patients with anti-NMDAR encephalitis did not affect memory (A), anxiety-related behavior (B) locomotor activity (C), or balance (D) in mice. Data are the means + S.E.M.
**Figure 6:**
Abundance of GFAP-positive astrocytes in the CA1 region of hippocampus of mice following 2 weeks’ administration of anti-NMDAR antibody solution or CSF of patients with anti-NMDAR encephalitis. Representative immunostaining images of the CA1 region of sham mouse (A, B), mouse treated with CSF from patients with anti-NMDAR encephalitis (C, D) and a mouse 4 days after pilocarpine-induced status epilepticus (E, F) at 10x and 40x, respectively.