BRAIN REPORT

NMDA-receptor-Fc-fusion constructs neutralize anti-NMDA receptor antibodies

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N-methyl-D-aspartate receptor (NMDAR) encephalitis is the most common subtype of autoimmune encephalitis characterized by a complex neuropsychiatric syndrome usually including memory impairment. Patients develop an intrathecal immune response against NMDARs with antibodies that presumably bind to the amino-terminal domain of the GluN1 subunit. The therapeutic response to immunotherapy is often delayed. Therefore, new therapeutic approaches for fast neutralization of NMDAR antibodies are needed. Here, we developed fusion constructs consisting of the Fc part of immunoglobulin G and the amino-terminal domains of either GluN1 or combinations of GluN1 with GluN2A or GluN2B. Surprisingly, both GluN1 and GluN2 subunits were required to generate high-affinity epitopes. The construct with both subunits efficiently prevented NMDAR binding of patient-derived monoclonal antibodies and of patient CSF containing high-titre NMDAR antibodies. Furthermore, it inhibited the internalization of NMDARs in rodent dissociated neurons and human induced pluripotent stem cell-derived neurons. Finally, the construct stabilized NMDAR currents recorded in rodent neurons and rescued memory defects in passive-transfer mouse models using intrahippocampal injections. Our results demonstrate that both GluN1 and GluN2B subunits contribute to the main immunogenic region of the NMDAR and provide a promising strategy for fast and specific treatment of NMDAR encephalitis, which could complement immunotherapy.

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Introduction

Since the initial description of anti-NMDAR (*N*-methyl-D-aspartate receptor) encephalitis in 2007,[1] the impact on neurology and psychiatry has been remarkable and has led to the definition of a new group of CNS disorders called 'autoimmune encephalitis'. These diseases are characterized by specific and pathogenic antineuronal antibodies directed at synaptic antigens. Patients with autoimmune encephalitis present with a variable pattern of severe neuropsychiatric symptoms that may lead to long-lasting coma within weeks.[2] Potential triggers for the autoimmune response include paraneoplastic molecular mimicry by ectopic expression of neuronal antigens in tumours, e.g. teratomata in NMDAR encephalitis, or antigens released by neuronal damage, e.g. after herpes simplex encephalitis. However, in most cases, no such triggers have been identified yet. The immune response in NMDAR encephalitis induces circulating B cells and intrathecally expanded antibody-producing cells in the brain.[3] The main pathomechanism is binding of the antibodies to the amino-terminal domain (ATD) of the NMDAR GluN1 subunit,[4,5] which causes clustering and internalization of NMDARs, most probably by cross-linking mechanisms.[6]

For the treatment of NMDAR encephalitis, no validated guidelines exist yet and the available immunotherapy shows limited efficacy (reviewed by Sell *et al.*[7]). About 25% of patients with NMDAR encephalitis are refractory to treatment[8] and often require long-term intensive care treatment due to life-threatening complications.[9] Antibody-depleting strategies (e.g. plasma exchange), B cell depletion (e.g. with rituximab) or experimental plasma cell targeting (e.g. with the proteasome inhibitors bortezomib[10,11]) have limited efficiency in the CNS compartment[12] and/or do not affect the main antibody-producing intrathecal plasma cells directly.[2] Furthermore, due to the half-life of immunoglobulin G (IgG) antibodies of ~20 days and long-living plasma cells in the CNS compartment, the therapeutic response to any immunotherapy is significantly delayed. These limitations of immunotherapy explain the often-prolonged recovery from disease symptoms and indicate that more specific and effective therapeutic approaches are needed.

The following three more specific approaches to interfere with direct effects of anti-NMDAR antibodies have been considered. First, the activation of the ephrinB2 receptor (EphB2R) by a soluble form of its ligand ephrin-B2 can stabilize NMDAR density[6] and rescue visuospatial learning[13] after pathogenic anti-NMDAR antibody application in mice. The underlying mechanism involves phosphorylation of the GluN2B subunit of the NMDAR on activation of the EphB2R,[14] which controls synaptic NMDAR clustering and retention. Second, positive allosteric modulators of NMDAR, such as 24(S)-hydroxycholesterol, were shown to potentiate the remaining, non-internalized NMDAR, thereby compensating for the NMDAR loss.[15] However, both approaches might overcompensate the loss of NMDARs and interfere with endogenous NMDAR function and membrane cycling. Third, monovalent Fab fragments were able to bind to NMDARs without inducing cross-linking, internalization or reduction of NMDAR density.[16] However, interference with Fab fragments to prevent binding of the antibodies is hampered by the greater avidity of IgG in comparison to Fab fragments, a potential pathogenic effect of Fab fragments by interfering with NMDAR function[16] and a shorter serum half-life of Fab fragment due to the protective function of the lacking Fc fragment.[17] Thus, the currently considered approaches to specifically treat NMDAR encephalitis have certain limitations.

To overcome these limitations, we developed an ATD-Fc-fusion construct that can neutralize pathogenic autoantibodies of patients while leaving NMDAR function unperturbed. We show that the construct prevents the binding of autoantibodies to the NMDAR, which represents the initial, disease-defining step. The constructs can therefore inhibit the hallmarks of the disease's pathophysiology including internalization of NMDARs, reduction of NMDAR currents and memory defects.

Materials and methods

Detailed methods are provided in the Supplementary material.

NMDAR encephalitis patients and CSF sample collection

CSF samples from NMDAR encephalitis patients (NMDAR antibody titre in CSF 1:100) were collected from Jena University Hospital. All the voluntary donors were informed about the project and gave their written consent. The use of CSF samples was approved by the ethical

committee of Jena University Hospital (approval # 2019-1415), the Technische Universität Braunschweig (Ethik-Kommission der Fakultät 2 der TU Braunschweig, approval # FV-2020-02), and the Leipzig University (approval # 313/19-Ik).

Animals

All animal procedures were in accordance with the European (EU Directive 2010/63/EU, Annex IV for animal experiments), national and Leipzig University guidelines. All animal procedures were approved in advance by the federal Saxonian Animal Welfare Committee (T01/21 for mice, T29/19 for rats).

Statistical analysis

For data in Figs 3A(ii), B(ii), 4B and C, non-parametric ANOVA (Kruskal–Wallis) tests revealed highly significant differences ($P < 0.001$). In the figures, the *P*-values of non-parametric *post hoc* tests (Dwass–Steel–Critchlow–Fligner pairwise comparisons) were provided. The calculations were performed with jamovi (www.jamovi.org).

Data availability

Data that support the findings of this study are available from the corresponding author on reasonable request.

Results

An engineered ATD-Fc-fusion construct has a high affinity for pathogenic anti-NMDAR antibodies

The production of stable epitopes for pathogenic anti-NMDAR autoantibodies is complicated by the hydrophobic nature of the NMDAR and the conformation-dependent epitope in the ATD of NMDARs.[4,18] We therefore designed several fusion constructs containing different parts of the ATD of NMDARs and a Fc fragment of mouse or human IgG (Fig. 1A). The ATD part contained either two GluN1 ATDs from amino acid (aa) 19 to 397, two GluN2B ATD from aa 25 to 395, or a combination of two GluN1 and two GluN2B ATD connected with specific 36-aa long linker peptides[20] (Fig. 1B). The last has the potential to assemble as a dimer of GluN1-GluN2B heterodimers as observed in the crystal structure of the entire ATD of NMDARs containing GluN1 and GluN2B (Fig. 1A).[19,21] Both constructs containing either a mouse or a human Fc part were soluble up to at least 0.7 mg/ml (≈4 µM) and had the expected size of ~150 kDa (Fig. 1C).

To test the binding capability of the constructs to pathogenic antibodies, we investigated their binding to a high-affinity monoclonal anti-NMDAR IgG isolated from a patient with NMDAR encephalitis (clone #003-102; see Kreye *et al.*[22]). Coating the monoclonal anti-NMDAR IgG on a microtitre plate for an ELISA assay, titrating the concentration of the constructs and subsequent immunostaining for bound constructs revealed the best binding interaction for the GluN1-GluN2B-Fc fusion construct with a ~100-fold higher affinity compared with GluN1 alone (Fig. 1D). Similar results were obtained with a construct in which the order of the ATDs was changed (GluN2B-GluN1-Fc; data not shown) or the GluN1 and GluN2A subunits were combined (GluN1-GluN2A-Fc; Fig. 1D). We selected the GluN1-GluN2B-Fc fusion construct as our leading molecule for further studies, because of a higher expression rate of GluN1-GluN2B-Fc compared to GluN1-GluN2A-Fc constructs. Binding affinity of the GluN1-GluN2B-Fc fusion construct to the monoclonal anti-NMDAR IgG (clone #003-102; see Kreye *et al.*[22]) was measured with biolayer interferometry (BLI, see the 'Materials and methods' section). The measured dissociation constant (K_D) was 29 nM, which is similar to the previously determined affinity of the binding of monoclonal anti-NMDAR IgG (003-102) to NMDARs ($c_{50} = 1.2$ µg/ml = 8 nM).[23] These data indicate that a pathogenic anti-NMDAR autoantibody binds to the engineered GluN1-GluN2B-Fc fusion construct with high affinity.

The ATD-Fc-fusion construct prevents binding of mono- and polyclonal pathogenic antibodies to NMDARs

To test the ability of the GluN1-GluN2B-Fc fusion construct to neutralize pathogenic antibodies, we tested its ability to prevent binding of pathogenic antibodies to NMDARs. As a first simple and robust approach, we used the recombinant ATDs of the GluN1-GluN2B-Fc fusion construct itself to serve as a NMDAR surrogate and coated it on a microtitre plate for an ELISA assay (Fig. 2A). A solution with a mixture of the pathogenic antibody with a constant concentration and varying concentrations of the fusion construct was added to the ELISA plate. After incubation, the plates were washed and the autoantibody binding to the immobilized fusion constructs was quantified. Adding increasing concentrations of the fusion construct prevented binding of the monoclonal anti-NMDAR IgG (clone #003-102; see Kreye *et al.*[22]) to the immobilized ATDs of the fusion constructs [Fig. 2A(ii)]. The complete neutralization of the pathogenic antibodies suggests that two fusion constructs can bind to an antibody (one per Fab part) without steric hindrance.

To investigate whether the construct also prevents binding of a broader repertoire of pathogenic IgGs to the ATD of the NMDAR, we repeated the analysis with clinically relevant CSF samples containing high titres of NMDAR autoantibodies of two different patients (Patients 1 and 2). Consistent with the prevented binding of the monoclonal IgG, the construct prevented binding of the patients' pathogenic antibodies to the recombinant ATDs [Fig. 2A(iii and iv)]. The half-maximal inhibitory effect was obtained at a concentration of 2.6 and 6.1 nM of the GluN1-GluN2B-Fc fusion construct for the CSF from Patients 1 and 2, respectively. The constructs prevented IgG binding specifically, because increasing concentrations of a control Fc fusion construct [Angiotensin converting enzyme 2 (ACE-2)-Fc, see the 'Materials and methods' section] did not affect IgG binding [Fig. 2A(ii–iv)]. Furthermore, control antibodies [Fig. 2A(ii)] and CSF from a control patient [Fig. 2A(iii and iv)] did not bind to the constructs.

We next tested the ability of the GluN1-GluN2B-Fc fusion construct to inhibit binding of pathogenic antibodies to synaptic NMDAR of fixed dissociated rat hippocampal neurons [Fig. 2B(i)]. The postsynaptic density of excitatory synapses was marked with anti-Homer1 antibodies and the NMDARs were stained with a monoclonal anti-NMDAR antibody [clone #003-102; see Kreye *et al.*[22]; Fig. 2B(ii)]. With increasing concentrations of the fusion construct, the binding of anti-NMDAR IgG to Homer1-positive synapses was prevented [Fig. 2B(iii)]. The half-maximal inhibitory effect was obtained at an 11-fold higher concentration of the construct compared with the concentration of the antibody. Identical analyses with CSF from Patient 1 demonstrated that the construct prevents binding of patients' pathogenic antibodies to synaptic NMDARs [Fig. 2B(iv)]. The half-maximal inhibitory effect was obtained at a concentration of 10.4 nM GluN1-GluN2B-Fc fusion construct. These data demonstrate that the GluN1-GluN2B-Fc fusion construct can inhibit

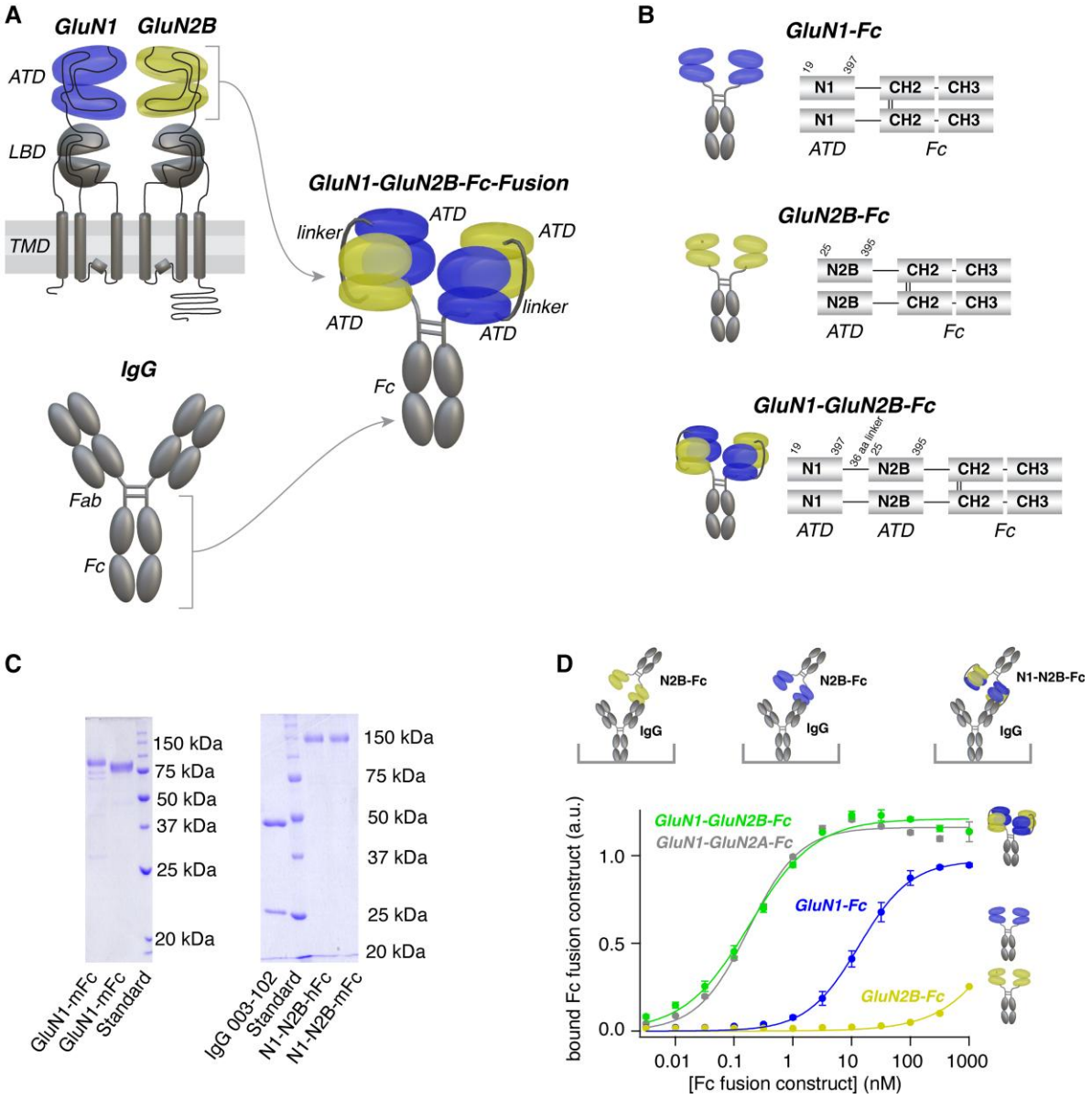

Figure 1 An engineered ATD-Fc-fusion construct has a high affinity for pathogenic anti-NMDAR antibodies. (**A**) Illustration of the GluN1 and GluN2B subunits of the NMDAR (modified from Chou *et al.*[19]), an IgG molecule and a fusion construct containing two ATDs of GluN1, two ATDs of GluN2B and an IgG Fc part. (**B**) Illustration of the three Fc-fusion constructs and their domains. (**C**) Sodium dodecyl sulphate–polyacrylamide gel electrophoresis with Coomassie staining of fusion constructs (mFc = mouse Fc; hFc = human Fc). (**D**) *Top*: An illustration of the fusion constructs GluN1-Fc (N1-Fc), GluN2B-Fc (N2B-Fc) and GluN1-GluN2B-Fc (N1-N2B-Fc) bound to IgG, which was coated on an ELISA microtitre plate. *Bottom*: The number of fusion constructs bound to monoclonal human IgG (003-102) plotted against the concentration of the fusion constructs containing the mouse Fc. In addition to the constructs illustrated on the *right*, the analysis of the GluN1-GluN2A-Fc fusion construct is shown in grey. The amount of the bound fusion constructs was detected with HRP conjugated secondary antibody against mouse Fc.

binding of mono- and polyclonal pathogenic antibodies to recombinant ATDs of NMDARs as well as synaptic NMDARs in hippocampal neurons.

The fusion construct prevents NMDAR internalization in rodent and human neurons

A hallmark of the pathophysiology of NMDAR encephalitis is the internalization of NMDARs due to binding of bivalent autoantibodies.[6] We therefore investigated NMDAR internalization in dissociated hippocampal neurons of rats [Fig. 3A(i)]. CSF from a patient was applied for 24 h to the culturing medium of the neurons. Subsequently, NMDARs on the cell surface were stained with immunocytochemistry (see the 'Materials and methods' section). The NMDAR signal within Homer1-postive excitatory synapses was reduced in a dose-dependent manner. Neurons treated with CSF from Patient 1 at a dilution of 1:20 and 1:100 reduce the NMDAR intensity to 22% and 43% compared to neurons treated with control CSF, respectively, indicating profound NMDAR internalization [red data in Fig. 3A(ii)]. Application of patient CSF and 100 nM of the GluN1-GluN2B-Fc fusion construct prevented receptor internalization [green data in Fig. 3A(ii);

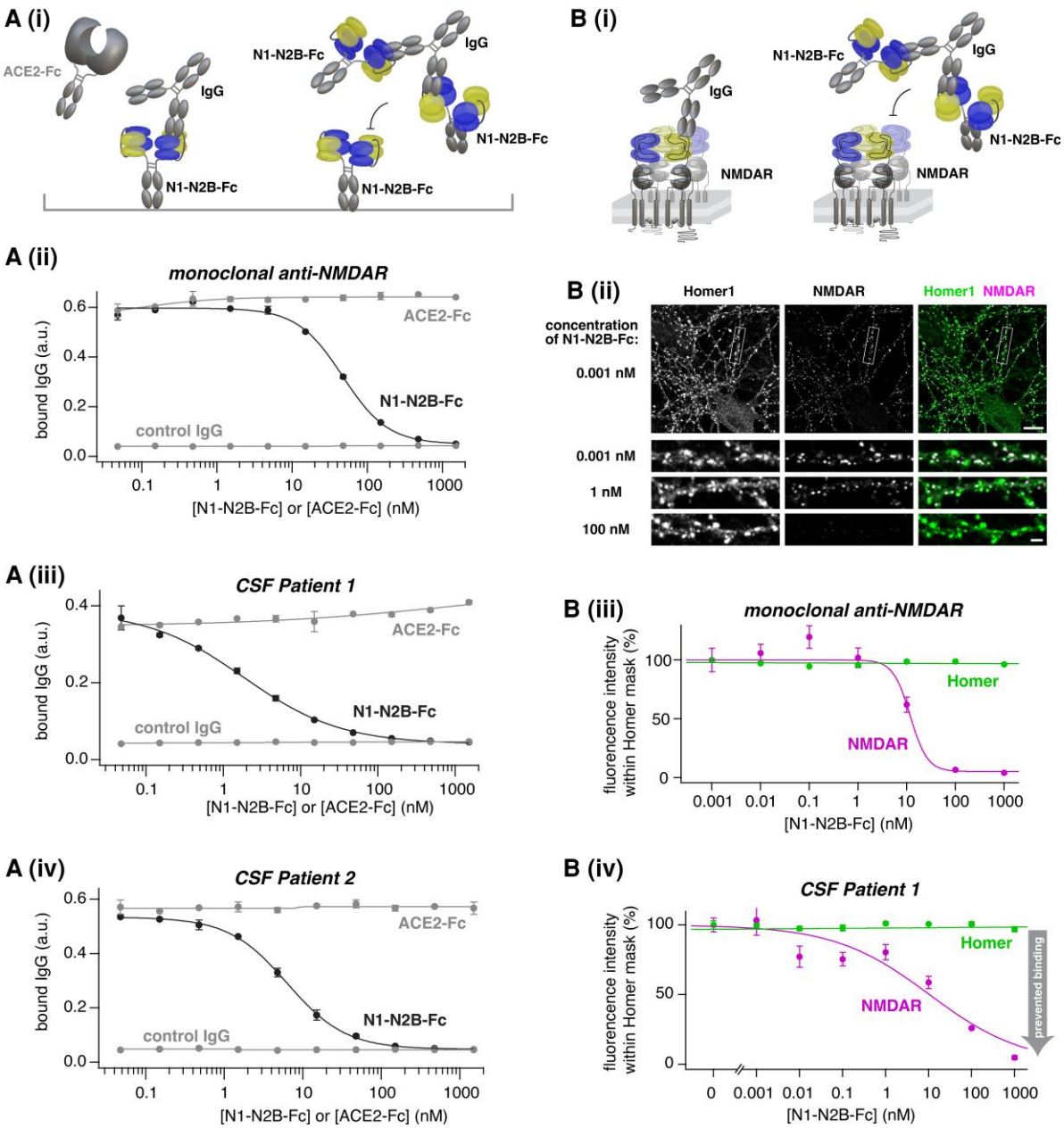

Figure 2 The ATD-Fc-fusion construct prevents binding of mono- and polyclonal pathogenic antibodies to NMDARs. [**A(i)**] Illustration of GluN1-GluN2B-Fc (N1-N2B-Fc) fusion construct coated on an ELISA microtitre plate with ACE-2-Fc in solution and bound IgG (*left*). In addition, an IgG neutralized by two N1-N2B-Fc fusion constructs is shown (*right*). [**A(ii)**] IgG (monoclonal 003-102) bound to the coated GluN1-GluN2B-Fc (N1-N2B-Fc) fusion constructs plotted against the concentration of the GluN1-GluN2B-Fc fusion construct in solutions (N1-N2B-Fc, black) or plotted against the concentration of a control fusion construct in solutions (ACE-2-Fc, grey). The solutions always contained 1.5 nM of the monoclonal IgG. Bound control IgG (palivizumab) is also shown (control IgG, grey). The amount of bound IgG was quantified by labelling with a secondary antibody (mean ± SEM of measured values per $n = 2$ wells of the titre plate is shown) [**A(iii)**] Same as in **A(ii)** but using CSF from Patient 1 and from a control patient. [**A(iv)**] Same as in **A(ii)** but using CSF from Patient 2 and from a control patient. [**B(i)**] Illustration of a NMDAR in a double lipid membrane of a neuron with bound IgG (*left*) and an IgG bound to two N1-N2B-Fc fusion constructs (*right*). [**B(ii)**] Example confocal fluorescence images of neurons stained for NMDARs (magenta) and Homer1 (green) treated for 24 h with CSF from Patient 1 (1:20) and 0.001, 1 or 100 nM of the GluN1-GluN2B-Fc (N1-N2B-Fc) fusion construct. Scale bar = 10 µm (*top row*) and 2 µm (*bottom three rows*). [**B(iii)**] Average fluorescence intensity within the Homer1 masks for stained NMDAR (magenta) and Homer1 (green) plotted against the concentration of the GluN1-GluN2B-Fc (N1-N2B-Fc) fusion construct in the solutions (mean ± SEM of the median fluorescence intensity per $n = 10$ images is shown; for each image, the median of the average pixel fluorescence of ~500 synapses was calculated). The solutions always contained 1 nM of the monoclonal anti-NMDAR IgG (003-102). [**B(iv)**] Same as in **B(iii)** but using CSF from Patient 1 (1:20) for binding and staining of NMDARs.

median 108 and 98% for 1:20 and 1:100 dilutions, respectively]. The fusion construct had no significant effect on the receptor density when applied for 24 hours with control CSF [grey data in Fig. 3A(ii)].

In addition, we repeated the NMDAR internalization assay in cultured neurogenin2-induced human induced pluripotent stem cell-derived excitatory neurons [Fig. 3B(i)].[24] Despite the low density of NMDAR receptors in these cultured human neurons,[24]

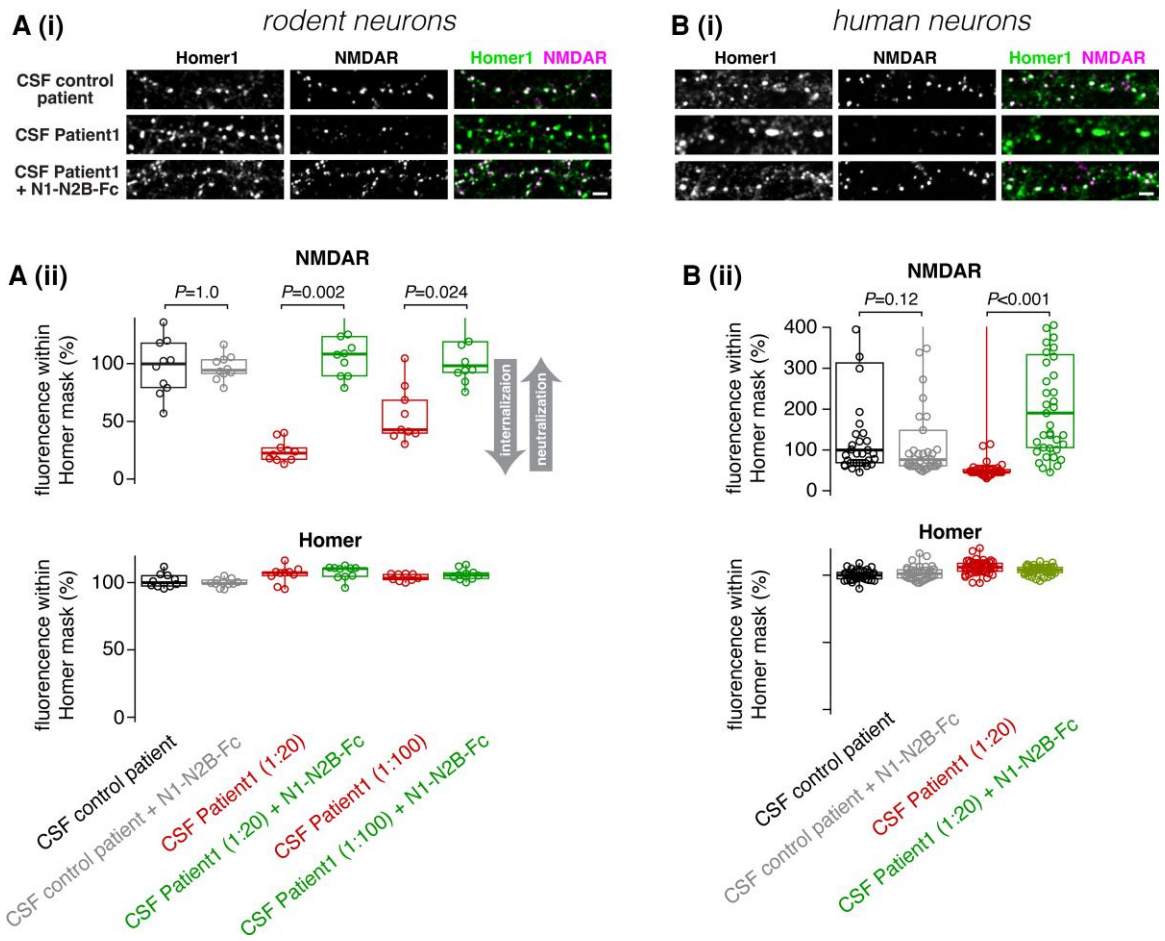

Figure 3 The fusion construct prevents NMDAR internalization in rodent and human neurons. [**A(i)**] Example confocal fluorescence images of neurons stained for NMDARs (magenta) and Homer1 (green) after 24 h incubation with control CSF at a 1:20 dilution (*top row*), CSF from Patient 1 at a 1:20 dilution (*middle row*) or CSF from Patient 1 at a 1:20 dilution and 100 nM of the N1-N2B-Fc fusion construct (*bottom row*). Scale bar = 2 µm. [**A(ii)**] Each data-point represents the median of the average fluorescence pixel intensity within ~500 Homer1-positive synapses per image for a staining against NMDARs (*top*) and Homer1 (*bottom*) of neurons treated for 24 h with control CSF at a 1:20 dilution (black), control CSF at a 1:20 dilution and 100 nM of the N1-N2B-Fc fusion construct (grey), CSF from Patient 1 at a 1:20 dilution (red), CSF from Patient 1 at a 1:20 dilution and 100 nM of the N1-N2B-Fc fusion construct (green), CSF from Patient 1 at a 1:100 dilution (red) and CSF from Patient 1 at a 1:100 dilution and 100 nM of the N1-N2B-Fc fusion construct (green). Data were normalized to the median value of neurons treated with control CSF. The indicated *P*-values are from Dwass–Steel–Critchlow–Fligner pairwise comparisons (see the 'Materials and methods' section). Note that some points are outside the plotted range. [**B(i)**] Corresponding example images of human induced pluripotent stem cell-derived neurons as shown in **A(i)**. Scale bar = 2 µm. [**B(ii)**] Corresponding analysis for human neurons as shown in **A(ii)**.

we resolved an NMDAR internalization on 24 h of incubation with patient CSF, which was inhibited by the GluN1-GluN2B-Fc fusion construct [Fig. 3B(ii)]. These data indicate that the fusion construct blocks receptor internalization triggered by autoantibodies of patients with NMDAR encephalitis in both rodent and human neurons.

The fusion construct stabilizes synaptic NMDAR currents and rescue memory defect

To functionally test the neutralization of pathogenic antibodies, we measured pharmacologically isolated spontaneous postsynaptic NMDAR currents in dissociated rat hippocampal neurons (Fig. 4A and B and Supplementary Fig. 1). In neurons treated with CSF from Patient 1 for 24 h, the postsynaptic currents were dramatically reduced compared with application of control CSF (Fig. 4B and C). In contrast, application of patient CSF and the fusion construct did not cause a significant reduction in the amplitude of postsynaptic currents (Fig. 4B and C). We observed an altered network activity in the cultured neurons treated with patient CSF, which could affect postsynaptic current amplitudes due to presynaptic vesicle depletion. We therefore repeated the analysis and analysed only the very largest current amplitude per cell and obtained similar results (Fig. 4D).

Finally, we investigated memory defects *in vivo* using a passive-transfer model and the novel object recognition test (Fig. 4E). Intrahippocampal injections of anti-NMDAR antibody (clone #003-102; see Kreye *et al.*[22]) prevented the otherwise significant preference for exploring a novel object compared with a known object (Fig. 4F; red data at Day 4). By contrast, intrahippocampal injections of anti-NMDAR antibody and the fusion construct did not impair the preference for the novel object (Fig. 4F; green data at Day 4). The injected fusion constructs diffused throughout the entire hippocampus (Supplementary Fig. 2), explaining its potent inhibition of the memory deficit. These data demonstrate that the newly developed GluN1-GluN2B-Fc fusion construct inhibited the

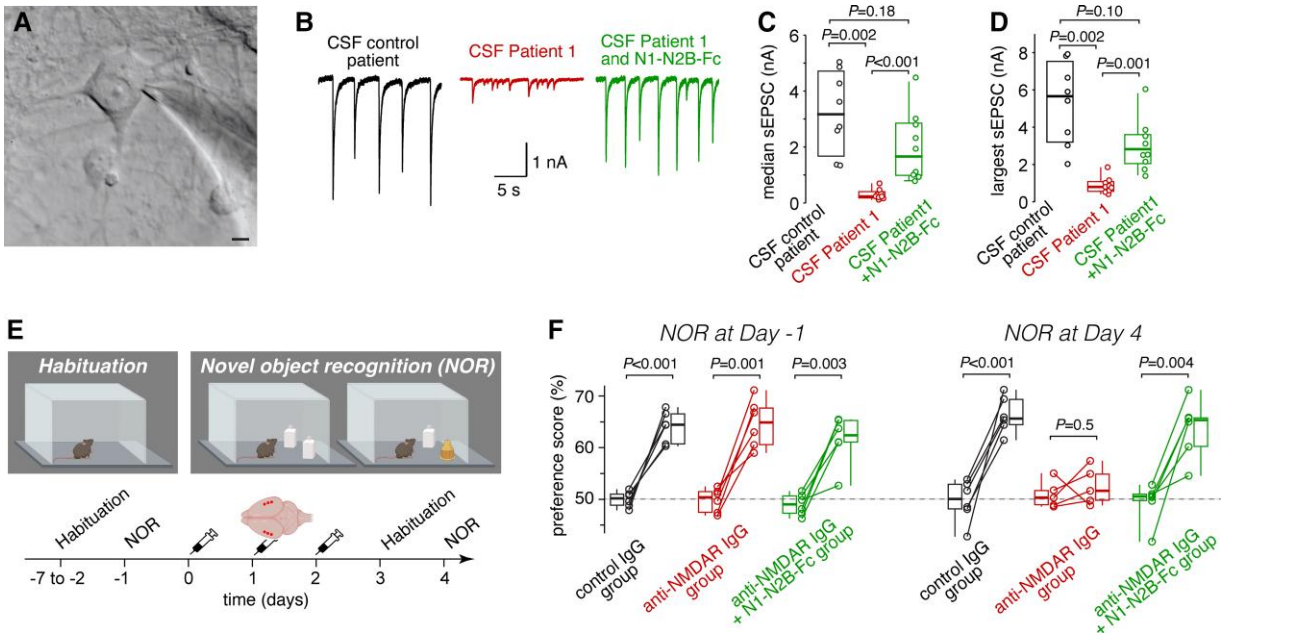

Figure 4 The fusion construct stabilizes synaptic NMDAR currents and rescue memory defect. (**A**) Example image of differential interference contrast (DIC) microscopy of a cultured hippocampal neuron (DIV 16) during whole-cell patch-clamp recording. Scale bar = 5 µm. (**B**) Examples of pharmacologically isolated spontaneous NMDAR currents of neurons incubated for 24 h with control CSF (black, *left*), CSF from Patient 1 at a 1:20 dilution (red, *middle*) and CSF from Patient 1 at a 1:20 dilution and 100 nM of the N1-N2B-Fc fusion construct (green, *right*). (**C**) Each data-point represents the median amplitude per neuron of the spontaneous excitatory postsynaptic currents (sEPSCs). The indicated *P*-values are from Dwass–Steel–Critchlow–Fligner pairwise comparisons (see the 'Materials and methods' section). Note that some points are outside the plotted range. (**D**) Corresponding analysis as in **C** but each data-point represents the very largest amplitude per neuron of the sEPSC. (**E**) Illustration of novel object recognition (NOR) test before and after intra-hippocampal injections at times of 0, 1 and 2 days. (**F**) Preference score for the novel object before (Day −1) and after (Day 4) injection of control IgG (black), anti-NMDAR IgG (red), anti-NMDAR IgG and N1-N2B-Fc fusion construct (green). The indicated *P*-values are from paired *t*-test without Bonferroni correction. Application of a Bonferroni correction with a factor of 6 leads to significant results in all comparisons ($P < 0.05$) except for the anti-NMDAR IgG group for novel object recognition at Day 4. All datasets passed the Shapiro–Wilk test for normal distribution (except for the control data of the anti-NMDAR IgG and N1-N2B-Fc fusion construct group at Day 4, but the corresponding non-parametric paired Wilcoxon W-test resulted in $P = 0.03$).

pathological reduction of synaptic NMDAR currents as well as resulting memory defects triggered by autoantibodies of patients suffering from NMDAR encephalitis.

Discussion

In this work, fusion constructs with ATDs of the NMDAR and IgG Fc parts were engineered. The construct containing the ATDs of both GluN1 and GluN2B subunits of the NMDAR bound pathogenic autoantibodies of patients with NMDAR encephalitis with high affinity. These constructs provide an exogenous target for the pathogenic antibodies prohibiting the binding of these antibodies to endogenous NMDAR receptors, thus serving as a 'baitbody' for pathogenic autoantibodies.

We here report a novel approach towards a rapid neutralization of antibodies in patients with NMDAR encephalitis that may also be combined with regular immunotherapy. In principle, such constructs could be used for direct and also repetitive intrathecal application in severely afflicted patients in the most active disease state during intensive care.[9] However, further studies are required to compare intrahippocampal and intrathecal applications of the constructs in mouse models of NMDAR encephalitis. Furthermore, additional studies are needed to test CSF or IgG preparations of more patients, because we investigated CSF of only two different patients. In the future, the use of bispecific, so-called 'brain-shuttle' antibodies[25] might also be an interesting option for systemic application thus overcoming the blood–brain barrier impermeability for macromolecules. Another therapeutic potential of the constructs could be a peripheral application for pregnant mothers with systemic anti-NMDAR autoantibodies, which might have transient or permanent pathogenic effects on CNS development of the foetus.[26]

The constructs developed here could also add to the panel of diagnostic tools to reliably detect CSF or serum antibodies to the NMDAR. The current routine diagnostic relies on cell-based assays and visual inspection. These tests are widely used in clinical practice and work very well but depend on the experience of the investigator and, in uncertain cases, additional and more specialized tests can be necessary to clarify antibody detection (e.g. brain section and live neuronal immunostaining).[27–29] A conformationally stable construct that can be applied easily in ELISA assays as demonstrated here might complement the routine cell-based assays and therefore contribute to robust, objective and fast detection of anti-NMDAR antibodies.

Our data provide novel insights into the pathophysiology of NMDAR encephalitis. We found that the ATD of GluN1 alone but not of the GluN2B alone provided an epitope for pathogenic antibodies. The importance of the GluN1 subunit for antibody binding is consistent with existing evidence that the pathogenic antibodies bind only to the GluN1 subunit.[4] Surprisingly, we found that the ATDs of both GluN1 and GluN2B (or GluN1 and GluN2A) were required for epitopes with the highest affinity. Our data thus suggest that either the high-affinity epitopes for pathogenic anti-NMDAR are composed of both subunits, or that the ATD of GluN1 increases its affinity or correct structural conformation by interaction with

GluN2A or GluN2B. However, our construct contains only the ATD of GluN1 but not the entire GluN1 subunit. Therefore, the increase in affinity on adding the ATD of GluN2A or GluN2B could be limited to ATDs isolated from the rest of the subunits. So far, it is not known whether full-length and conformationally intact GluN1 or the interaction of GluN1 with GluN2A or GluN2B is necessary to generate epitopes of high affinity. Consistent with both possibilities, recent immunization models using either conformationally stabilized and fully assembled tetrameric GluN1/GluN2B NMDARs[18] or ATD GluN1 peptides[30] resulted in fulminant encephalitis or rather mild disease symptoms, respectively.

In summary, we tested our developed fusion construct in a series of assays that reflect the critical steps of disease pathophysiology. Our data provide, to the best of our knowledge, the first therapeutic tool to efficiently and specifically neutralize pathogenic autoantibodies of patients suffering from NMDAR encephalitis that does not interfere with NMDAR function itself. The here-established therapeutic option of providing an antigen on an IgG Fc part (baitbody) could be used to neutralize antibodies against other antigens causing autoimmune encephalitis.[2]

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Competing interests

H.P. has a patent application pending for the use of similar NMDAR-Fc chimeras in autoimmune diseases.

Supplementary material

Supplementary material is available at *Brain* online.

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