ALLOSTERIC MODULATION OF NMDA RECEPTORS PREVENTS THE ANTIBODY EFFECTS OF PATIENTS WITH ANTI-NMDA RECEPTOR ENCEPHALITIS

Francesco Mannara[1]*, Marija Radosevic[1]*, Jesús Planagumà[1]*, David Soto[1,2], Esther Aguilar[1], Anna García-Serra[1], Estibaliz Maudes[1], Marta Pedreño[1], Steven Paul[3,4], James Doherty[3], Michael Quirk[3], Jing Dai[3], Xavier Gasull[1,2], Mike Lewis[3#], and Josep Dalmau.[1,5,6#]

[1]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Universitat de Barcelona, Barcelona, Spain

[2]Laboratori de Neurofisiologia, Departament de Biomedicina, Facultat de Medicina i Ciències de la Salut, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain

[3]Sage Therapeutics, Cambridge, MA, US

[4]Departments of Psychiatry and Neurology, Washington University School of medicine, St Louis, US

[5]Department of Neurology, University of Pennsylvania, Philadelphia, US

[6]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

*These authors contributed equally; [#]these authors share seniority

Corresponding author: Josep Dalmau, MD, PhD, IDIBAPS-Hospital Clínic, Universitat de Barcelona, Department of Neurology, c/ Villarroel 170, 08036 Barcelona, Spain, Phone: +34 93 227 1783; e-mail: jdalmau@clinic.cat

Running head: Modulation of NMDAR in encephalitis

Key words: Animal model; anti-NMDAR encephalitis; SGE-301; treatment

Number of words in abstract: 400; body of the manuscript: 4686. Number of figures: 5; Suppl material: text and 7 figures.

ABSTRACT (word limit 400, now 400)

Anti-N-Methyl-D-Aspartate Receptor (NMDAR) encephalitis is an immune-mediated disease characterized by a complex neuropsychiatric syndrome in association with an antibody-mediated decrease of NMDAR. About 85% of patients respond to immunotherapy (and removal of an associated tumor if it applies), but it often takes several months or more than 1 year for patients to recover. There are no complementary treatments, beyond immunotherapy, to accelerate this recovery. Previous studies showed that SGE-301, a synthetic analog of 24(S)-hydroxycholesterol, which is a potent, and selective positive allosteric modulator of NMDAR, reverted the memory deficit caused by phencyclidine (a non-competitive antagonist of NMDAR), and prevented the NMDAR dysfunction caused by patients' NMDAR antibodies in cultured neurons. An advantage of SGE-301 is that it is optimized for systemic delivery such that plasma and brain exposures are sufficient to modulate NMDAR activity. Here, we used SGE-301 to confirm that in cultured neurons it prevented the antibody-mediated reduction of receptors, and then we applied it to a previously reported mouse model of passive cerebroventricular transfer of patients' CSF antibodies. Four groups were established: mice receiving continuous (14-day) infusion of patients' or controls' CSF, treated with daily subcutaneous administration of SGE-301 or vehicle (no drug). The effects on memory were examined with the novel object location (NOL) test at different time points, and the effects on synaptic levels of NMDAR (assessed with confocal microscopy) and plasticity (long-term potentiation [LTP]) were examined in the hippocampus on day 18, which in this model corresponds to the last day of maximal clinical and synaptic alterations. As expected,

mice infused with patients' CSF antibodies, but not those infused with controls' CSF, and treated with vehicle developed severe memory deficit without locomotor alteration, accompanied by a decrease of NMDAR clusters and impairment of LTP. All antibody-mediated pathogenic effects (memory, synaptic NMDAR, LTP) were prevented in the animals that were treated with SGE-301, despite that this compound did not antagonize antibody binding. Additional investigations on the potential mechanisms related to these SGE-301 effects showed that (1) in cultured neurons SGE-301 prolonged the decay time of NMDAR-dependent spontaneous excitatory postsynaptic currents suggesting a prolonged open time of the channel, and (2) it significantly decreased the internalization of antibody-bound receptors suggesting that additional, yet unclear mechanisms, contribute in keeping unchanged the surface NMDAR density. Overall, these findings suggest that SGE-301, or similar modulators of NMDAR, could potentially serve as complementary treatment for anti-NMDAR encephalitis and deserve future investigations.

Abbreviations: anti-NMDA receptor (NMDAR), blood-brain-barrier (BBB), long-term potentiation (LTP), $\Delta^{5,6}$-3β-oxy-nor-cholenyl-dimethylcarbinol (SGE-301), novel object location (NOL)

INTRODUCTION

Anti-NMDA receptor (NMDAR) encephalitis is an immune-mediated disease characterized by a complex neuropsychiatric syndrome and the presence of CSF antibodies against the GluN1 subunit of NMDARs (Dalmau *et al.*, 2008). The disorder can be triggered by systemic tumors, usually a teratoma of the ovary, and less frequently by herpes simplex encephalitis (Armangue *et al.*, 2018), but in many cases no trigger is identified. At disease onset patients develop psychosis, insomnia, abnormal movements, seizures, decreased level of consciousness, dysautonomia, or coma, which

in about 85% of cases respond to immunotherapy and removal of the tumor when it applies (Titulaer *et al.*, 2013; Viaccoz *et al.*, 2014). However, it often takes several months or more than 1 year for patients to return to most of their activities. During the process of recovery the clinical features are different from those of the acute stage, including impairment of attention, memory, executive functions, or behavior (Dalmau *et al.*, 2011; Finke *et al.*, 2012; Peer *et al.*, 2017). The reasons for this slow clinical recovery are unclear but may include a persisting immune activation against NMDAR within the CNS, a severe impairment of synaptic function and long-term plasticity, a limited blood-brain barrier (BBB) penetration of current immunotherapies, or a combination of these factors. Studies examining the effects of patients NMDAR antibodies in cultured neurons (Hughes *et al.*, 2010; Mikasova *et al.*, 2012) or mice (Planaguma *et al.*, 2015; Planaguma *et al.*, 2016) have shown that they mediate a broad loss of surface NMDARs, regardless of synaptic localization or subunit composition (Warikoo *et al.*, 2018), leading to impairment of synaptic plasticity and memory (Planaguma *et al.*, 2015; Planaguma *et al.*, 2016).

In some respects, the treatment paradigm of anti-NMDAR encephalitis resembles that of antibody-mediated diseases of the neuromuscular junction, such as myasthenia gravis or the Lambert-Eaton myasthenic syndrome (LEMS), where despite evidence that several immunotherapies are effective, most patients need additional treatment for a faster or sustained improvement. These treatments are addressed to compensate or overcome the mechanisms altered by the autoantibodies, for example, acetylcholinesterase inhibitors (pyridostigmine) in myasthenia gravis, or the presynaptic potassium channel blocker (3,4-diaminopyridine) in the LEMS (Newsom-Davis, 2003; Wirtz *et al.*, 2010). In studies using cultured neurons (Mikasova *et al.*, 2012; Planaguma *et al.*, 2016) or passive transfer of patients' CSF NMDAR antibodies to mice (Planaguma *et al.*, 2016), a soluble form of ephrin-B2 (an agonist of the ephrin-B2 receptor which clusters and retains NMDARs at the synapse) was able to antagonize all antibody-mediated effects including NMDAR internalization and impairments of long-term plasticity and visuospatial

memory. As a proof of principle, this finding showed that interfering with the antibody-mediated mechanisms could potentially be used as a complementary treatment with immunotherapy (Mikasova *et al.*, 2012); however, ephrin-B2 was administered intraventricularly and there are no available ephrin-B2 agonists that cross the BBB.

There is evidence that a major brain-derived cholesterol metabolite, 24(S)-hydroxycholesterol (24(S)-HC), is a very potent, direct, and selective positive allosteric modulator (PAM) of NMDARs (Paul *et al.*, 2013). In hippocampal slices, application of 24(S)-HC enhanced the ability of subthreshold stimuli to induce long-term potentiation (LTP), and reversed the LTP deficits caused by the NMDAR channel blocker, ketamine. Several synthetic analogues of 24(S)-HC such as $\Delta^{5,6}$-3β-oxy-nor-cholenyl-dimethylcarbinol (or SGE-201) or SGE-301 shared similar mechanisms of action (Paul *et al.*, 2013). In rats, the administration of SGE-301 reverted the memory deficit caused by phencyclidine, a non-competitive NMDAR antagonist (Paul *et al.*, 2013). Moreover, application of SGE-301 to cultures of neurons exposed to CSF antibodies from patients with anti-NMDAR encephalitis prevented the antibody-mediated dysfunction of NMDARs (Warikoo *et al.*, 2018). An advantage of this compound is that it is optimized for systemic delivery such that plasma and brain exposures are sufficient to modulate activity in pre-clinical models of NMDAR hypofunction (Paul *et al.*, 2013). These findings led us to determine whether SGE-301 was able to prevent the antibody-mediated reduction of NMDARs and impairment of memory observed in a reported model of cerebroventricular transfer of patients' CSF antibodies.

MATERIALS AND METHODS

Animals, surgery, and patients' CSF

Seventy-six male C57BL/6J mice (Charles River), 8-10 weeks old (25-30 g) were used for the studies including, memory and locomotor activity (47 mice), confocal immunohistochemistry assessment of levels of NMDAR and other synaptic proteins, and electrophysiological studies (29 mice). Animal care, anesthesia, insertion of bilateral ventricular catheters (PlasticsOne, model 3280PD-2.0; coordinates: 0.2 mm posterior and ± 1.00 mm lateral from bregma, depth 2.2 mm), and connection of each catheter to a subcutaneous osmotic pump for continuous infusion of CSF (Alzet, Cupertino, CA, US; volume 100 µl, flow rate 0.25 µl/h for 14 days) have been reported (Planaguma *et al.*, 2015). The CSF infused was pooled from samples of 10 patients with high titer IgG GluN1 antibodies (all > 1:320), and 10 patients with normal pressure hydrocephalus without NMDAR antibodies (control samples).

The presence of NMDAR antibodies in patients' CSF (and absence in controls' CSF) was examined with three different techniques, brain tissue immunohistochemistry, HEK293T cells expressing NMDAR, and cultured neurons, as reported (Ances *et al.*, 2005; Dalmau *et al.*, 2008). Patients' and controls' CSF were then pooled in two different samples and filtered (Amicon Ultracel 30K, Sigma-Aldrich, St Louis , MI, US), dialyzed against phosphate-buffered saline (PBS), and normalized to a physiologic concentration of 2 mg IgG/dL (Planaguma *et al.*, 2016). The absence of other antibodies in pooled patients' CSF was confirmed using an aliquot immunoabsorbed with HEK293T expressing GluN1, showing: (1) abrogation of reactivity with mouse brain and HEK293T cells expressing NMDARs, and (2) abrogation of NMDAR internalization (Supplementary Fig. 1).

Written informed consent was obtained from all patients. The study was approved by the local institutional review board (Hospital Clínic, HCB/2018/0192), and animal studies were approved by the Local Ethical Committee of the University of Barcelona following European (2010/63/UE) and Spanish (RD 53/2013) regulations about the use and care of experimental animals.

Preparation and treatment with SGE-301

SGE-301 is a potent allosteric modulator of the NMDA receptor that has been characterized previously (Paul *et al.*, 2013). For the current studies, we adopted a subcutaneous administration paradigm (vs. intraperitoneal) to minimize interaction with the centrally fixed osmotic minipumps. Therefore, we ran plasma and brain pharmacokinetic studies to measure exposures of SGE-301 present at the time of *in vivo* testing. The method of determination of plasma and brain concentration of SGE-301 is described in supplementary information. At 1 hour, we achieved 1954 ± 157 ng/ml plasma and 523 ± 86 ng/g brain exposures. At 4 hours, we achieved 985 ± 173 ng/ml plasma and 1350 ± 120 ng/g brain exposures (Supplementary Fig. 2). These exposures are similar to those reported after intraperitoneal administration (Paul *et al.*, 2013).

For studies with cultured neurons, lyophilized SGE-301 was weighted and dissolved in dimethyl sulfoxide (DMSO) to a stock concentration of 10 mM; the solution was then sonicated for 1 hour at 40°C and used at a working concentration of 10 μM. For studies using mice, lyophilized SGE-301 was weighted and dissolved in a solution of 30% 2-hydroxypropyl-β-cyclodextrin (HPBCD, Sigma-Aldrich) in distilled water. The solution was then vortexed for 5 minutes, sonicated for 40 minutes, and stirred for 2 hours at 50°C. After adjusting the pH to 5.5-7.0, working aliquotes were prepared and kept frozen at -20°C. A similar solution of 30% HPBCD in distilled water, but without drug, served as control (vehicle). Aliquots with or without drug were thawed and vortexed for 2 minutes before use.

Experimental design

Four experimental groups were established including mice infused with patients' or controls' CSF along with subcutaneous administration of SGE-301 (10 mg/kg) or vehicle (Fig. 1A). The administration of SGE-301 started on day 1 (coinciding with day 1 of infusion of CSF) until day

18 (four days after the infusion of CSF had stopped) which is the last day with maximal memory deficits and reduction of NMDAR synaptic clusters observed in this model (Planaguma *et al.*, 2015). The selected animal tasks (novel object location; locomotor activity) and the timing of the tasks (Fig. 1B) were based on previous experience with this model, showing that patients' CSF NMDAR antibodies caused a progressive decrease of visuospatial memory until day 18, subsequently followed by progressive recovery several days after the antibody infusion stopped (Planaguma *et al.*, 2015). In contrast, patients' antibodies did not significantly alter the locomotor activity (included here as control, and to ensure that animals did not have motor limitations in exploring the objects). All tasks were performed by researchers blinded to experimental conditions.

Immunohistochemistry and confocal microscopy

Techniques related to immunolabeling of live cultures of dissociated rodent hippocampal neurons,[2] immunoabsorption of patients' samples with GluN1-expressing HEK cells, brain tissue processing, and quantitative brain tissue immunoperoxidase staining, have been previously reported (Planaguma *et al.*, 2015). To determine the effects of patients' antibodies in cultured rat hippocampal neurons, 17-day *in vitro* cultures were exposed to patients' or controls' CSF (diluted 1:100) along with 10 μM SGE-301 or vehicle for 24 hours, and the cell surface clusters of NMDAR, PSD95, and their colocalization (representing synaptic NMDAR) were quantified with specific biomarkers and confocal microscopy (Supplementary information). The effect of 48-hour exposure to SGE-301 on the surface clusters of NMDAR and levels of phosphorylated PSD95 (phospho-S295-PSD95) in cultured neurons was determined using a similar immunocytochemical technique and specific phospho-S295-PSD95 antibodies (Supplementary information). Determination of antibody-bound internalized NMDAR was performed as reported (Moscato *et al.*, 2014) (Supplementary information).

To determine the effects of patients' antibodies on the number of clusters of NMDAR and PSD95, non-permeabilized 5 μm-thick brain sections (obtained at day 18, Fig. 1B) were blocked with 5% goat serum, and serially incubated with a human CSF NMDAR-antibody sample (1:20, used as primary antibody) for 2 hours at room temperature (RT) and the secondary Alexa Fluor 488 goat anti-human IgG (1:1000, A-11013, ThermoFisher, Waltham, MA, US) for 1 hour at RT. Tissue sections were then permeabilized with 0.3% Triton X-100 for 10 minutes at RT, and serially incubated with rabbit polyclonal anti-PSD95 (1:250, ab18258 Abcam, Cambridge , UK) overnight at 4°C, and the corresponding secondary Alexa Fluor 594 goat anti-rabbit IgG (1:1000, A-11012, ThermoFisher) for 1 hour at RT. Slides were then mounted with ProLong Gold antifade reagent for 4 minutes, containing 6-diamidino-2-phenylindole dihydrochloride (DAPI, P36935; ThermoFisher) and results scanned with Zeiss LSM710 confocal microscope (Carl Zeiss, Jena, Germany) with EC-Plan NEOFLUAR CS 100×/1.3 NA oil objective. For each animal, five identical image stacks in three hippocampal areas (CA1, CA3 and dentate gyrus; total 15 image stacks) were acquired as reported (Planaguma *et al.*, 2015). Each *z*-stack comprised 50 optical images that were deconvolved with AutoQuantX3 (Bitplane, Oxford Instruments, Abingdon, UK). The mean density of clusters of NMDAR or PSD95 was obtained using a spot detection algorithm from Imaris suite 7.6.4 (Bitplane), and the cluster density expressed as spots/mm^3. The clusters of NMDAR that colocalized with PSD95 were defined as synaptic. For each experimental group, the mean cluster densities of NMDAR or PSD95 were normalized with the corresponding values in control animals (infused with controls' CSF and treated with vehicle).

To determine the levels of synaptic phospho-S295-PSD95 in brain tissue, 5 μm-thick brain sections permeabilized as above and blocked with 5% goat serum and 1% bovine serum albumin (BSA) were incubated with rabbit anti-phospho-S295-PSD95 (1:200, ab76108, Abcam) and mouse anti-PSD95 (diluted 1:200, 124 011, Synaptic Systems, Goettingen, Germany) for 1 hour. Slides were then washed and incubated with Alexa Fluor 488 goat anti-rabbit IgG and Alexa Fluor

594 goat anti-mouse IgG (both diluted 1:500, A-11034, A-11032, ThermoFisher). Results were scanned as above, and the cluster density of phospho-S295-PSD95 and PSD95 was determined with Imaris (Bitplane) software.

Electrophysiological studies

Preparation of acute hippocampal slices on day 18 (Fig. 1B) and field potential recordings and analysis were performed as reported (Planaguma et al., 2016) (Supplementary information).

To determine the effects of chronic exposure to SGE-301 on NMDAR currents we treated 18 d.i.v. cultures of hippocampal neurons with controls' CSF (diluted 1:100) or controls' CSF + SGE-301 (10mM) for 48h prior to whole-cell patch clamp recordings of spontaneous NMDAR-mediated excitatory postsynaptic currents (sEPSCs) (Supplementary information).

Memory and locomotor activity tasks

Visuospatial memory was assessed with the novel object location (NOL) discrimination index, and the locomotor activity was automatically determined using locomotor activity boxes (11x21x18 cm, Imetronic, Passac, France) for 1 hour (Planaguma *et al.*, 2015) (Supplementary information).

Statistical analysis

Data from behavioral studies (novel object location and locomotor activity) were analyzed using repeated-measures two-way ANOVA. Human IgG intensities from different brain regions and confocal clusters densities of NMDAR and PSD95 on cultured neurons and brain tissue were analyzed using one-way ANOVA. Density levels of phospho-S295-PSD95 in cultured neurons and brain tissue were assessed with unpaired *t-test*. The electrophysiological data was assessed by one-way ANOVA (long-term potentiation and paired-pulse facilitation: PP2/PP1 ratios) and

unpaired *t-test* (paired-pulse facilitation: analysis of increase of PP2 slope compared with PP1 in each of the experimental groups independent of each other). A p value < 0.05 was considered significant. All ANOVA tests included *post-hoc* analyses with Bonferroni correction for multiple testing. Analysis of NMDAR-mediated sEPSC in cultures of neurons chronically exposed to SGE-301 was performed with Student's *t*-tests. Statistical analyses were performed with GraphPad Prism v.6 (La Jolla, CA, US).

RESULTS

Treatment with SGE-301 prevents the pathogenic effects of antibodies in cultured neurons

We and others previously reported that patients' NMDAR antibodies cause a reduction of the clusters of synaptic and extrasynaptic NMDARs in cultured neurons (Hughes *et al.*, 2010; Mikasova *et al.*, 2012), and in an animal model of cerebroventricular infusion of patients' CSF (Planaguma *et al.*, 2015). Here, we first used cultured neurons to determine whether the antibody effects were prevented by SGE-301. As expected, neurons treated with patients' CSF and vehicle showed a significant decrease of total cell surface and synaptic NMDAR clusters compared with neurons treated with controls' CSF and vehicle; however, neurons treated with the same patients' CSF antibodies along with SGE-301, instead of vehicle, showed no significant change of the levels of total cell surface or synaptic NMDARs (Fig. 2). To determine whether this was due to abrogation of receptor internalization, we quantified the clusters of antibody-bound internalized NMDAR (Moscato *et al.*, 2014) showing that treatment with SGE-301 significantly reduced the levels of internalized antibody-bound receptors, but did not completely abolished the internalization (supplementary Fig 3). These findings show that in neurons exposed to patients' antibodies, treatment with SGE-301 prevents the reduction of cell-surface NMDAR. Current data

suggest that this treatment effect results from a reduction of antibody-mediated internalization of receptors and additional, yet unclear mechanisms, which overall keep the clusters of surface receptors similar to control levels.

Neurons treated for 24 hours with controls' CSF and SGE-301 showed a mild decrease of synaptic NMDAR clusters. To further explore the cause of this decrease of synaptic NMDAR we examined the effects of a longer (48-hours) neuronal exposure to SGE-301, which demonstrated a decrease of synaptic and extrasynaptic NMDAR clusters (supplementary Fig 4A). Considering that phosphorylation of ser-295 enhances the accumulation of PSD95 and that ser-295 phosphorylation is suppressed by chronic NMDAR activation (Kim *et al.*, 2007), we determined whether SGE-301 changed the levels of phospho-S295-PSD95. This experiment showed a reduction of phospho-S295-PSD95 without significant decrease total PSD95 (supplementary Fig 4B). A similar reduction of phospho-S295-PSD95 was obtained when cultured neurons were incubated with bicuculline, as reported (Kim *et al.*, 2007), and used here as control (Supplementary Fig. 4C). These findings indicate that prolonged neuronal exposure to SGE-301 leads to a reduction of NMDARs accompanied by a decrease of phospho-S295-PSD95 suggesting the presence of compensatory changes to the positive modulation of NMDAR.

To determine the effects of chronic exposure to SGE-301 on NMDAR currents we treated hippocampal neuronal cultures with controls' CSF (diluted 1:100) or controls' CSF + SGE-301 (10mM) for 48 hours prior to whole-cell patch clamp recordings of spontaneous NMDAR-mediated sEPSCs. Recordings revealed that SGE-301 did not modify the amplitude or frequency of sEPSC (Supplementary Figure 5) but significantly slowed the decay phase of the sEPSC, as shown by a longer decay time constant (262.0±37.3 ms *vs.* 368.6±49.8 ms; $p<0.05$; Supplementary Fig 5B). These findings suggest that SGE-301 enhances NMDAR-mediated EPSCs by slowing their decay phase, most probably by increasing the channel's open time and thus decreasing NMDAR's deactivation time.

Treatment with SGE-301 prevents the antibody-mediated reduction of NMDAR in mice

We next assessed whether SGE-301 antagonized the antibody effects in the hippocampus of mice infused with patients' CSF antibodies. Fifteen hippocampal areas with 50 optical *z*-sections per area, representing 750 optical sections per animal (five animals per experimental group), were investigated (Fig. 3A). Animals infused with patients' CSF and treated with vehicle showed a significant decrease of the density of total and synaptic NMDAR clusters compared with animals infused with controls' CSF and treated with vehicle or SGE-301. Similarly as observed with cultured neurons, the pathogenic effect of patients' CSF antibodies was prevented in the group of animals that received the same patients' CSF but were treated with SGE-301 instead of vehicle (Fig. 3B, C). To assess whether the treatment effect of SGE-301 was due to a direct interference with patients' antibody binding to NMDARs, we determined the intensity of human IgG bound to hippocampus in mice representative of the four experimental groups. This study showed that SGE-301 did not modify the intensity of patients' CSF IgG present in hippocampus suggesting that the drug did not block the binding of the antibody to NMDARs (Supplementary Fig. 6).

An additional finding of these studies was that in control conditions (e.g., animals not infused with antibodies) chronic administration of SGE-301 caused a decrease of total cell surface and synaptic NMDAR clusters as well as a decrease of PSD95, as shown comparing the groups of animals treated with controls' CSF with or without SGE-301 (Fig. 3C). Moreover, the hippocampus of mice infused with controls' CSF and chronically treated with SGE-301 had a significant decrease of ser-295 phosphorylated PSD95 and total PSD95 compared with animals not treated with SGE-301 (Supplementary Fig. 7). Overall, these studies showed that subcutaneous administration of SGE-301 abolished the antibody-mediated reduction of synaptic and extrasynaptic clusters of NMDARs, and that in control conditions (animals not infused with

NMDAR antibodies) SGE-301 led to a decrease of levels of NMDAR and PSD95 suggesting, as with the experiments with neurons, the presence of compensatory mechanisms to the positive modulatory effect of SGE-301 on NMDARs.

Treatment with SGE-301 prevents the impairment of LTP caused by patients' NMDAR antibodies

Acute brain slices from mice infused with patients' or controls' CSF treated with SGE-301 or vehicle, were used to record field excitatory postsynaptic potentials (fEPSPs) in the CA1 region of the hippocampus (Fig. 4A). Animals infused with patients' CSF showed a significant reduction of long-term potentiation (LTP) compared with animals infused with controls' CSF, as shown by analysis of fEPSP slope change (Fig. 4B, C). Quantitative analysis showing median changes in slope values during the stable period post-theta-burst stimulation (TBS) (from minutes 15 to 60, of 60 minutes after TBS) showed a reduced potentiation of fEPSP in mice infused with patients' CSF compared with those infused with controls' CSF (Fig. 4D). Treatment with SGE-301 prevented patients' CSF antibody-mediated impairment of LTP (Fig. 4C, D). Compared with these findings, animals infused with controls' CSF, with or without treatment with SGE-301, did not show impairment of LTP, although the control group treated with SGE-301 showed a non-significant reduction of fEPSP slope change (Fig. 4C). This finding probably reflects the decreased density of NMDAR clusters noted in the confocal analysis of effects of SGE-301 in animals infused with controls' CSF (Fig. 3C).

In contrast to the severe reduction of hippocampal LTP, short-term plasticity was not affected in animals infused with patients' CSF antibodies, as expected from the experience with previous studies (Planaguma *et al.*, 2016). Indeed, fEPSP recordings following a standard paired pulse

protocol showed significant facilitation consistent with increased presynaptic release probability (Supplementary Fig. 8A,B). This effect was similar in the four experimental groups (Supplementary Fig. 8C). Overall, these studies showed a severe impairment of postsynaptic, but not presynaptic, plasticity after TBS in animals infused with patients' CSF, but not in animals infused with the same patients' CSF and simultaneously treated with SGE-301.

Treatment with SGE-301 prevents memory loss caused by patients' NMDAR antibodies

Mice infused with patients' CSF and treated with daily subcutaneous administration of vehicle developed a progressive decrease of the NOL index, with maximal deficit on days 10 and 18 (four days after stopping the antibody infusion), followed by progressive memory improvement until reaching the baseline pre-infusion level on day 25 (pink line in Fig. 5) (Planaguma *et al.*, 2015). In contrast, mice infused with the same patients' CSF but treated with daily subcutaneous injections of SGE-301 instead of vehicle, showed no alteration of the NOL index (blue line, Fig. 5); these findings were similar to those of mice infused with controls' CSF treated with SGE-301 or vehicle (light and dark green lines, Fig. 5). The total time of exploration of the two objects (not moved + novel location) was similar in animals of the four experimental groups (Supplementary Fig. 9A). The locomotor activity was also similar in the four groups of animals (Supplementary Fig. 9B-D). Overall, these findings showed that daily subcutaneous administration of SGE-301 prevented the hippocampal memory impairment caused by patients' NMDAR antibodies in this animal model.

DISCUSSION

In this proof of concept study we show that a synthetic analogue (SGE-301) of the brain-derived cholesterol metabolite 24(S)-HC prevented the pathogenic effects of antibodies from patients with anti-NMDAR encephalitis in hippocampal neuronal cultures and in a previously reported model of cerebroventricular transfer of antibodies (Planaguma *et al.*, 2015; Planaguma *et al.*, 2016). These findings and the good brain concentration after subcutaneous dosing suggest that oxysterol-based NMDAR PAMs could serve as potential treatments for anti-NMDAR encephalitis.

Like steroids, oxysterols are well-recognized signaling molecules that interact with membrane-bound as well as soluble intracellular receptors (Radhakrishnan *et al.*, 2007). In particular, 24-hydroxylated oxysterol, such as 24 (S)-HC and the synthetic analogues, SGE-201 and SGE-301, are known for a striking selectivity for NMDARs (Paul *et al.*, 2013; Linsenbardt *et al.*, 2014). The enzyme involved in synthesis of 24(S)-HC (cholesterol 24-hydroxylase; CYP46A1) is expressed predominantly in the endoplasmic reticulum of neurons and dendrites (Ramirez *et al.*, 2008) and its deficiency causes severe impairment of hippocampal LTP and memory in mice (Kotti *et al.*, 2006). Using slices of hippocampus of rats, previous studies showed that application of 24(S)-HC or synthetic oxysterols (SGE-201 or SGE-301) reversed the LTP inhibition caused by ketamine (a non-competitive antagonist of NMDAR) (Paul *et al.*, 2013). In rats, the impairment of memory and active social interactions caused by phencyclidine (PCP), a non-competitive antagonist of NMDARs, were significantly improved by SGE-301 (Paul *et al.*, 2013). These findings, together with results from our studies, suggest that SGE-301 prevents the NMDAR hypofunction caused by pharmacological antagonists as well as by immune-mediated mechanisms.

Studies with chimeric GluN-GluK subunits suggest that GluN transmembrane domains are critical for oxysterol modulation (Wilding *et al.*, 2016) which would be consistent with the lipophilic nature of these modulators. SGE-301 increases channel open probability, potentiating NMDAR function, and appears to bind to a site independent of other allosteric modulators of

NMDAR function (Paul *et al.*, 2013; Wilding *et al.*, 2016). In a previous study in which cultured rat hippocampal neurons were exposed for 48 hours to patients' CSF NMDAR antibodies or controls' CSF and during the last 24 hours each condition was treated with SGE-301 or vehicle, those that were treated with SGE-301 showed increased NMDAR function compared with those treated with vehicle (Warikoo *et al.*, 2018). Similar to our observed prolonged sEPSCs duration after SGE-301 (Fig S5), an increase of NMDAR function was reported also in the neurons exposed to controls' CSF and treated with SGE-301, which has been attributed to an increase in open probability of the NMDA receptor (Warikoo et al, 2018). This lead the authors to suggest that this compound does not interfere directly with patients' antibody-mediated internalization of NMDARs (Warikoo *et al.*, 2018). This interpretation was limited by the fact that the authors did not consider that the maximal antibody-mediated reduction of NMDARs in cultured neurons occurs within the first 12-24 hours-incubation (before SGE-301 was applied); afterwards, the clusters of NMDARs remain decreased for as long as the antibodies are present in the media (Moscato *et al.*, 2014; Ladepeche *et al.*, 2018). Our current data show that when patients' CSF antibodies were co-applied with SGE-301 to cultures of neurons, the expected antibody-mediated effects were abolished, and the clusters of NMDAR were not decreased. Similar findings occurred in the animal model, showing that the density of total cell surface and synaptic NMDARs clusters in mice infused with patients' CSF and treated with SGE-301 was not significantly different from that of control mice (infused with controls' CSF and treated with vehicle). In contrast, animals infused with patients' CSF but not treated with SGE-301 showed the expected significant reduction of NMDARs. This reduction of NMDAR was associated with severe impairment of LTP and visuospatial memory, which were prevented when animals were simultaneously treated with SGE-301.

We noted that mice infused with control CSF and treated with SGE-301 compared with mice infused with the same control CSF and treated with vehicle, showed a decrease of total cell surface

and synaptic NMDAR clusters. A similar effect was noted in neuronal cultures treated for 48 hours with controls' CSF and SGE-301. We postulated that this finding represents a compensatory mechanism to the chronic PAM activity of SGE-301. Studies have shown that phosphorylation of ser-295 enhances the accumulation of PSD95 and that phospho-S295-PSD95 is suppressed by chronic NMDAR activation (Kim *et al.*, 2007). In line with these studies, we found that mice infused with controls' CSF and chronically treated with SGE-301 had lower amounts of phospho-S295-PSD95 and PSD95 compared with mice treated with vehicle. Neuronal cultures treated for 48 hours with SGE-301 showed an effect in the same direction, including a reduction of phospho-S295-PSD95 that was more intense than that of total PSD95. Similar mechanisms induced by the chronic PAM effect of SGE-301 may be involved in the change, although not significant, in fEPSP slope after induction of LTP in control animals treated with SGE-301 compared with those treated with vehicle.

Data from this and a previous study (Planaguma *et al.*, 2016) show that patients' antibodies do not affect paired-pulse facilitation, suggesting that presynaptic neurotransmitter release is unaffected in all experimental groups and that post-synaptic mechanisms are responsible for the decrease of LTP in animals treated with patients' CSF. In another report using hippocampal neuronal cultures, patients' antibodies specifically decreased NMDAR-mediated currents without affecting AMPA receptor-mediated currents (Hughes *et al.*, 2010). These studies along with the selective SGE-301 PAM effect on NMDAR (Paul *et al.*, 2013) suggest that the impairment of LTP (and its prevention by SGE-301) in animals infused with patients' CSF is via modulation of NMDAR.

The exact molecular mechanisms by which SGE-301 prevents the effects of patients' antibodies are unknown. We found that SGE-301 does not block the binding of patients' antibodies to hippocampus, suggesting several alternative mechanisms such as interference with antibody-induced internalization of receptors, increase of recruitment of NMDARs, or both. In preliminary

studies with cultured neurons treated with patients' antibodies, we found that SGE-301 significantly decreased (without fully abrogating) antibody-mediated NMDAR internalization, suggesting that in this setting a recruitment of NMDARs to the cell surface and synapse may be facilitated by the drug.

Our study design does not allow assessing whether SGE-301 reverses the antibody-mediated decrease of NMDAR and associated memory deficit because animals infused with patients' antibodies were simultaneously treated with SGE-301, and they did not develop any of those alterations. Although previous studies showed that application of SGE-301 to neuronal cultures exposed to patients' antibodies for 24 hours accelerated the recovery from the antibody effects (Warikoo *et al.*, 2018), it is unclear if SGE-301 would fully reverse symptoms already established and if so, how long it would take to recovery. It is also unclear whether SGE-301 would be effective for symptoms other than memory impairment; animal models reproducing the entire repertoire of symptoms in the acute and chronic stage of the disease would facilitate these studies. Finally, there is evidence that SGE-301 and similar PAMs potentiate the NMDAR responses for many minutes beyond their presence in the media, a feature attributed to their strong lipophilicity or potential intracellular accumulation (Paul *et al.*, 2013; Warikoo *et al.*, 2018). Therefore, a dosing less frequent than that used in our model (e.g., every other day instead of daily dosing) may result in the same beneficial effects.

The experience with current treatment approaches to anti-NMDAR encephalitis and the outcome of most patients emphasize the importance of our findings. During the acute stage of anti-NMDAR encephalitis patients often require intensive immunotherapy, antiepileptics, psychoactive medications, and intensive care support, along with tumor removal if this applies (Titulaer *et al.*, 2013). This stage is usually followed by a protracted process of recovery in which symptoms of the acute phase (psychosis, seizures, abnormal movements, decreased level of consciousness) are no longer present, and the patient is at home or in a rehabilitation center

showing other symptoms such as deficit of memory, attention, cognition, abnormal behavior, or executive dysfunction (Finke *et al.*, 2012; Finke *et al.*, 2013; Titulaer *et al.*, 2013). Our model, in which the local transfer of human NMDAR antibodies into mice cerebroventricular system predominantly affects hippocampal NMDAR (Planaguma *et al.*, 2015), provides a proof of principle that targeting the antibody-related mechanisms as complementary treatment for anti-NMDAR encephalitis, may mitigate or shorten the process of recovery. In preliminary studies, SAGE-718 (a PAM closely related to SGE-301 designed for oral bioavailability and once daily dosing) showed a good tolerability profile in healthy volunteers in a double-blind, placebo-controlled phase 1 single ascending dose study (Koenig *et al.*, 2019) and is currently being used in a trial for Huntington disease (which at early stages appears to associate with reduced NMDAR function). The tasks for the future are to better understand the underlying mechanisms by which SGE-301 prevents patients' antibody effects, assess the ability of this compound to reverse established symptoms, and determine its optimal dosing and frequency of treatment.

ACKNOWLEDGEMENTS

We thank Esther Aguilar, Mercedes Alba and Eva Caballero (IDIBAPS, Hospital Clinic, University of Barcelona) for their technical support.

FUNDING

This study was funded by Plan Nacional de I+D+I and cofinanced by the ISCIII – Subdirección General de Evaluación y Formento de la Investigación Sanitaria – and the Fondo Europeo de Desarrollo Regional (ISCIII-FEDER; 17/00234 and 17/00296); Project Integrative of Excellence (PIE 16/00014); CIBERER (#CB15/00010); RETICs Oftared RD16/0008/0014 (X.G.); "La Caixa" Foundation (ID 100010434, under the agreement LCF/PR/HR17/52150001); The Safra

Foundation (JD), and Fundació CELLEX (JD); BFU2017-83317-P (D.S.); Pla Estratègic de Recerca i Innovació en Salut (PERIS, SLT002/16/00346, J.P); and FI-AGAUR grant program by the Generalitat de Catalunya (2019FI_B1 00212, A.G-S) and Maria de Maeztu MDM-2017-0729 to Institut de Neurociencies.

CONFLICTS OF INTEREST

Dr. Dalmau receives royalties from Athena Diagnostics for the use of Ma2 as an autoantibody test and from Euroimmun for the use of NMDA as an antibody test. He received a licensing fee from Euroimmun for the use of $GABA_B$ receptor, $GABA_A$ receptor, DPPX and IgLON5 as autoantibody tests; he has received a research grant from Sage Therapeutics. Drs. Steven Paul, James Doherty, Michael Quirk, Jing Dai, and Mike Lewis work at Sage Therapeutics.

LEGENDS TO FIGURES

FIG. 1: Experimental design

A) Four experimental groups of mice were used, including mice treated with continuous cerebroventricular infusion of controls' or patients' CSF for 14 days along with daily subcutaneous injection of vehicle (30% HPBCD) or SGE-301 (10 mg/kg diluted in vehicle) for 18 days.

B) Timing of memory and locomotor tasks. Novel object location (NOL) and locomotor activity (LA) tests were started before the surgical implantation of ventricular catheters and osmotic pumps. The same tests were applied on days 3-4, 10-11, 18-19 and 25-26 after surgery. The effects of patients' antibodies on the levels of NMDARs and synaptic plasticity were examined on subsets of mice sacrificed on day 18, which is the date of maximal effects reported in this model (Planaguma *et al.*, 2015).

FIG. 2: Treatment with SGE-301 prevents the reduction of NMDARs caused by patients' antibodies in cultured neurons

Panel A: Representative dendrites of hippocampal neurons immunostained for surface NMDAR (green) and PSD95 (red) after 24 hour-treatment with patients' CSF or controls' CSF, each with either vehicle or SGE-301. Synaptic NMDARs are defined as those that colocalize with PSD95 (white channel). Scale bars= 10 μm.

Panel B: Quantification of the density of surface and synaptic NMDAR. Cultures co-treated with patients' CSF antibodies and vehicle showed a significant decrease of total cell surface and synaptic NMDARs without affecting the density of PSD95. In contrast, cultures co-treated with the same patients' CSF and SGE-301 did not show reduction of NMDARs. No effects on total cell surface NMDARs were noted in neurons treated with controls' CSF with vehicle or SGE-301, although the presence of SGE-301 associated with a mild reduction of synaptic NMDARs. The density of PSD95 was not affected by any of these conditions. N= 15 dendrites per condition, three independent experiments. Box plots show the median, and 25th and 75th percentiles; whiskers indicate the minimum and maximum values. Significance of treatment effect was assessed by one-way ANOVA ($p < 0.0001$ for NMDAR, synaptic NMDAR) with Bonferroni *post-hoc* correction: *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$.

FIG. 3: Treatment with SGE-301 prevents the reduction of NMDARs caused by patients' antibodies in hippocampus

Panel A: Hippocampus of mouse immunolabeled for NMDAR and PSD95. Images were merged (synaptic NMDAR, yellow color) and post-processed to demonstrate colocalizing clusters. White

squares indicate the analyzed areas in CA1, CA3, and dentate gyrus. Each square is a three-dimensional (3D) stack of 50 sections. Scale bar= 500 μm.

Panel B: 3D projection and analysis of the density of total cell surface NMDAR clusters, PSD95, and synaptic NMDAR clusters (defined as those that colocalized with PSD95). Each 3D projection is a representative CA1 square region (as those shown in panel A) of an animal representative of each experimental condition infused with controls' or patients' CSF along with SGE-301 or vehicle. Merged images (merge: PSD95 [red]/NMDAR [green]) were post-processed and used to calculate the density of clusters (density= spots/μm^3). Scale bar= 2 μm.

Panel C: Quantification of the density of total (left) and synaptic (right) NMDAR clusters, and total PSD95 at day 18 in a pooled analysis of hippocampal areas (CA1, CA3, and dentate gyrus). Mean density of clusters in animals treated with controls' CSF + vehicle was defined as 100%. For each condition, five animals were examined (15 hippocampal areas per animal). Box plots show the median, and 25th and 75th percentile; whiskers indicate the minimum or maximum values. Significance of treatment effect was assessed by one-way ANOVA analysis of variance (p= 0.0001) and *post-hoc* analysis with Bonferroni correction; ***$p < 0.001$; ****$p < 0.0001$.

FIG. 4: Treatment with SGE-301 prevents the impairment of LTP caused by patients' NMDAR antibodies

Panel A: The Schaffer collateral pathway (SC, red) was stimulated (Stim) and field potentials were recorded (Rec) in the CA1 region of the hippocampus. Long-term potentiation (LTP) was induced by theta-burst stimulation (TBS); DG= dentate gyrus; CA= Cornu Ammonis.

Panel B: Example traces of individual recordings showing baseline fEPSPs before LTP induction (black traces) and after LTP (red traces). Slope and peak amplitude of fEPSPs are increased after TBS in mice infused with controls' CSF and treated with vehicle or SGE-301, and are strongly

impaired in animals infused with patients' CSF and treated with vehicle. In mice infused with patients' CSF and treated with SGE-301 the increase of slope is improved. Note that initial peak amplitude of fEPSP may vary within individual recordings.

Panel C: Time course of fEPSP recordings demonstrating robust changes in fEPSP slope in the animals infused with controls' CSF treated with vehicle (n = eight recordings from seven animals, light green), or treated with SGE-301 (n = 10 recordings from seven animals, dark green) which is stable throughout the recording period after TBS (arrow). In animals infused with patients' CSF and treated with vehicle (n = six recordings from five animals, pink) the induction of LTP is markedly impaired. In contrast, animals infused with the same patients' CSF and treated with SGE-301 (n= seven recordings from six animals, blue) show resolved effects on synaptic plasticity after LTP induction. The fEPSP values of all animals for each of the groups are presented as mean ± SEM.

Panel D: Quantification of fEPSP slope change showing a significant reduction of fEPSP slope in animals infused with patients' CSF and treated with vehicle compared with the other groups of animals. Note that animals infused with patients' CSF and treated with SGE-301 did not show reduction of fEPSP slope. The number of recordings and animals used are the same as those indicated in panel C. Box plots show the median, 25^{th} and 75^{th} percentiles; whiskers indicate minimum and maximum values. Significance was assessed by one-way ANOVA ($p < 0.01$) and Bonferroni *post-hoc* correction test was applied: *$p < 0.05$; **$p < 0.01$.

FIG. 5: SGE-301 prevented the visuospatial memory deficits caused by patients' NMDAR antibodies

Mice infused with patients' CSF antibodies and treated with vehicle (pink line) showed a significant reduction of the NOL index. This memory deficit was prevented in the group of mice

infused with the same patients' CSF antibodies but treated with SGE-301 (blue line). No significant memory changes were noted in the groups of mice infused with controls' CSF and treated with vehicle (light green line) or SGE-301 (dark green line). Number of animals: controls' CSF + vehicle = 11; patients' CSF + vehicle = 10; controls' CSF + SGE-301 = 12; patients' CSF + SGE-301 = 10. A higher index represents better visuospatial memory. Data are presented as mean ± SEM. Significance of assessment was performed by repeated-measures two-way analysis of variance (ANOVA; $p < 0.0001$) with Bonferroni *post-hoc* correction. Patients' CSF + vehicle versus controls' CSF + vehicle: $^{****}p < 0.0001$, $^{***}p < 0.001$. Patients' CSF + vehicle versus patients' CSF + SGE-301: $^{\$\$\$}p < 0.001$.

REFERENCES

Ances BM, Vitaliani R, Taylor RA, Liebeskind DS, Voloschin A, Houghton DJ*, et al.* Treatment-responsive limbic encephalitis identified by neuropil antibodies: MRI and PET correlates. Brain 2005; 128(Pt 8): 1764-77.

Armangue T, Spatola M, Vlagea A, Mattozzi S, Carceles-Cordon M, Martinez-Heras E*, et al.* Frequency, symptoms, risk factors, and outcomes of autoimmune encephalitis after herpes simplex encephalitis: a prospective observational study and retrospective analysis. Lancet Neurol 2018; 17(9): 760-72.

Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M*, et al.* Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 2008; 7(12): 1091-8.

Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. Lancet Neurol 2011; 10(1): 63-74.

Finke C, Kopp UA, Pruss H, Dalmau J, Wandinger KP, Ploner CJ. Cognitive deficits following anti-NMDA receptor encephalitis. J Neurol Neurosurg Psychiatry 2012; 83(2): 195-8.

Finke C, Kopp UA, Scheel M, Pech LM, Soemmer C, Schlichting J*, et al.* Functional and structural brain changes in anti-N-methyl-D-aspartate receptor encephalitis. Ann Neurol 2013; 74(2): 284-96.

Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R*, et al.* Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J Neurosci 2010; 30(17): 5866-75.

Kim MJ, Futai K, Jo J, Hayashi Y, Cho K, Sheng M. Synaptic accumulation of PSD-95 and synaptic function regulated by phosphorylation of serine-295 of PSD-95. Neuron 2007; 56(3): 488-502.

Koenig A, Murck H, Paskavitz J, Hoffmann E, Li S, Silber C*, et al.* Double-blind, placebo-controlled phase 1 single ascending dose study of SAHE-718. International College of Neuropsychopharmacology (CINP) 2019 Congress. Athens, Greece; 2019.

Kotti TJ, Ramirez DM, Pfeiffer BE, Huber KM, Russell DW. Brain cholesterol turnover required for geranylgeraniol production and learning in mice. Proc Natl Acad Sci U S A 2006; 103(10): 3869-74.

Ladepeche L, Planaguma J, Thakur S, Suarez I, Hara M, Borbely JS*, et al.* NMDA receptor autoantibodies in autoimmune encephalitis cause a subunit-specific nanoscale redistribution of NMDA receptors. Cell Rep 2018; 23(13): 3759-68.

Linsenbardt AJ, Taylor A, Emnett CM, Doherty JJ, Krishnan K, Covey DF*, et al.* Different oxysterols have opposing actions at N-methyl-D-aspartate receptors. Neuropharmacology 2014; 85: 232-42.

Mikasova L, P. DR, Bouchet D, Georges F, Rogemond V, Didelot A*, et al.* Disrupted surface cross-talk between NMDA and Ephrin-B2 receptors in anti-NMDA encephalitis. Brain 2012; 135(Pt 5): 1606-21.

Moscato EH, Peng X, Jain A, Parsons TD, Dalmau J, Balice-Gordon RJ. Acute mechanisms underlying antibody effects in anti-N-methyl-D-aspartate receptor encephalitis. Ann Neurol 2014; 76(1): 108-19.

Newsom-Davis J. Therapy in myasthenia gravis and Lambert-Eaton myasthenic syndrome. Semin Neurol 2003; 23(2): 191-8.

Paul SM, Doherty JJ, Robichaud AJ, Belfort GM, Chow BY, Hammond RS*, et al.* The major brain cholesterol metabolite 24(S)-hydroxycholesterol is a potent allosteric modulator of N-methyl-D-aspartate receptors. J Neurosci 2013; 33(44): 17290-300.

Peer M, Pruss H, Ben-Dayan I, Paul F, Arzy S, Finke C. Functional connectivity of large-scale brain networks in patients with anti-NMDA receptor encephalitis: An observational study. Lancet Psychiatry 2017; 4(10): 768-74.

Planaguma J, Haselmann H, Mannara F, Petit-Pedrol M, Grunewald B, Aguilar E*, et al.* Ephrin-B2 prevents N-methyl-D-aspartate receptor antibody effects on memory and neuroplasticity. Ann Neurol 2016; 80(3): 388-400.

Planaguma J, Leypoldt F, Mannara F, Gutierrez-Cuesta J, Martin-Garcia E, Aguilar E*, et al.* Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. Brain 2015; 138(Pt 1): 94-109.

Radhakrishnan A, Ikeda Y, Kwon HJ, Brown MS, Goldstein JL. Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig. Proc Natl Acad Sci U S A 2007; 104(16): 6511-8.

Ramirez DM, Andersson S, Russell DW. Neuronal expression and subcellular localization of cholesterol 24-hydroxylase in the mouse brain. J Comp Neurol 2008; 507(5): 1676-93.

Titulaer MJ, McCracken L, Gabilondo I, Armangue T, Glaser C, Iizuka T*, et al.* Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. Lancet Neurol 2013; 12(2): 157-65.

Viaccoz A, Desestret V, Ducray F, Picard G, Cavillon G, Rogemond V*, et al.* Clinical specificities of adult male patients with NMDA receptor antibodies encephalitis. Neurology 2014; 82(7): 556-63.

Warikoo N, Brunwasser SJ, Benz A, Shu HJ, Paul SM, Lewis M*, et al.* Positive allosteric modulation as a potential therapeutic strategy in anti-NMDA receptor encephalitis. J Neurosci 2018; 38(13): 3218-29.

Wilding TJ, Lopez MN, Huettner JE. Chimeric Glutamate Receptor Subunits Reveal the Transmembrane Domain Is Sufficient for NMDA Receptor Pore Properties but Some Positive Allosteric Modulators Require Additional Domains. J Neurosci 2016; 36(34): 8815-25.

Wirtz PW, Titulaer MJ, Gerven JM, Verschuuren JJ. 3,4-diaminopyridine for the treatment of Lambert-Eaton myasthenic syndrome. Expert Rev Clin Immunol 2010; 6(6): 867-74.

FIGURE 1

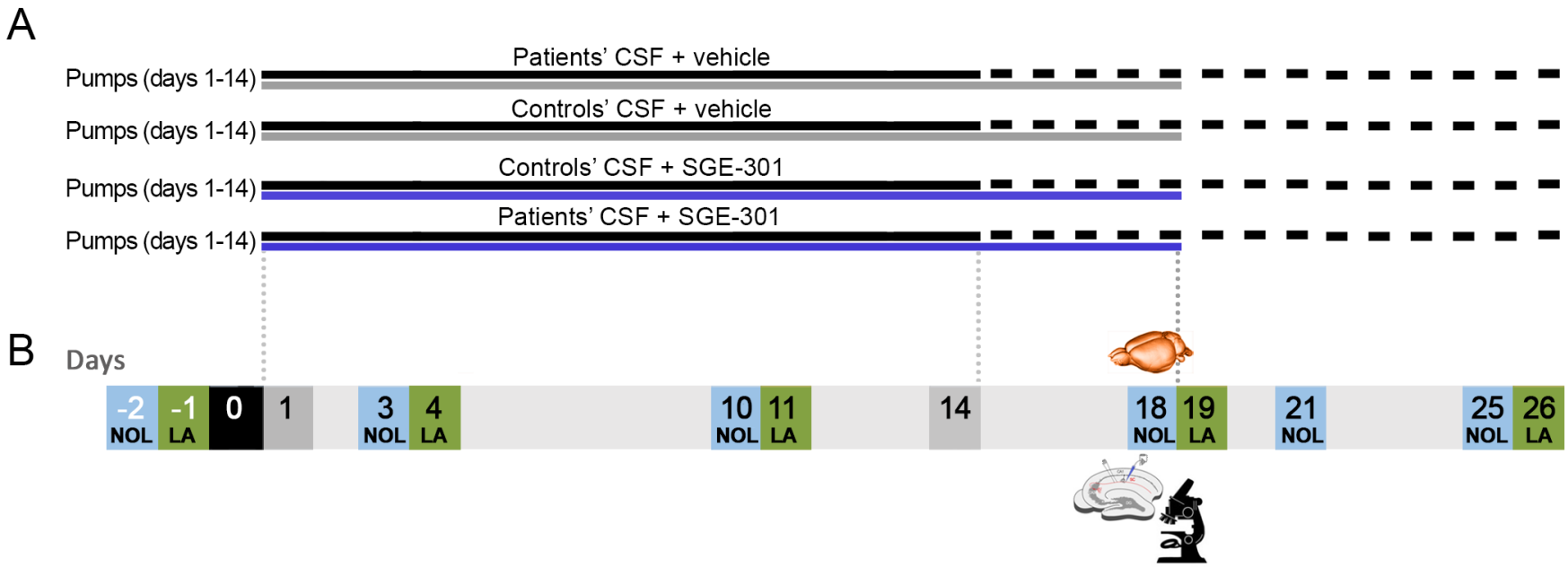

A
Patients' CSF + vehicle
Pumps (days 1-14)
Controls' CSF + vehicle
Pumps (days 1-14)
Controls' CSF + SGE-301
Pumps (days 1-14)
Patients' CSF + SGE-301
Pumps (days 1-14)
B
Days
-2 NOL
-1 LA
0
1
3 NOL
4 LA
10 NOL
11 LA
14
18 NOL
19 LA
21 NOL
25 NOL
26 LA

FIGURE 2

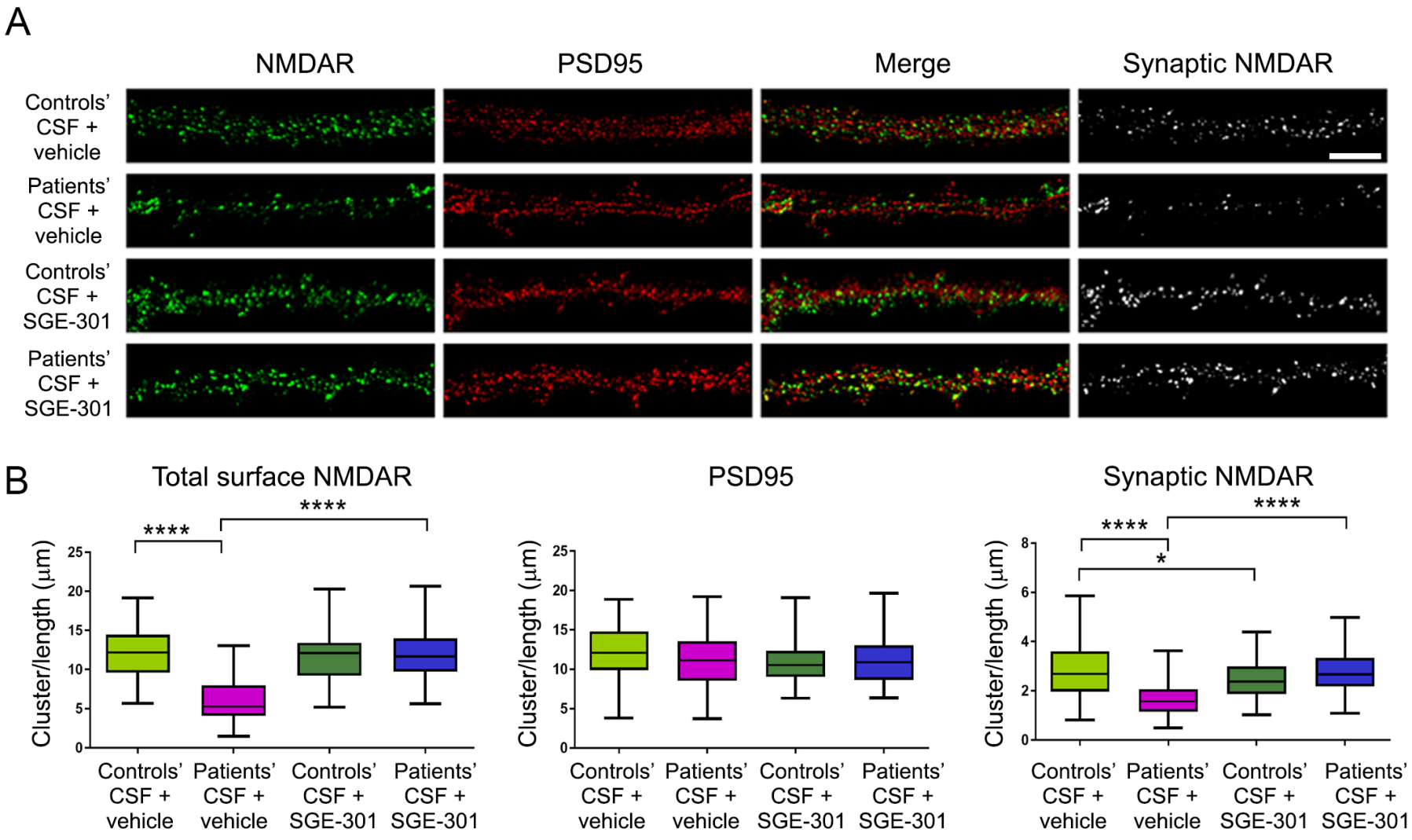

A
NMDAR
PSD95
Merge
Synaptic NMDAR
Controls' CSF + vehicle
Patients' CSF + vehicle
Controls' CSF + SGE-301
Patients' CSF + SGE-301
B
Total surface NMDAR
PSD95
Synaptic NMDAR
Cluster/length (µm)
****
*
Controls' CSF + vehicle
Patients' CSF + vehicle
Controls' CSF + SGE-301
Patients' CSF + SGE-301

FIGURE 3

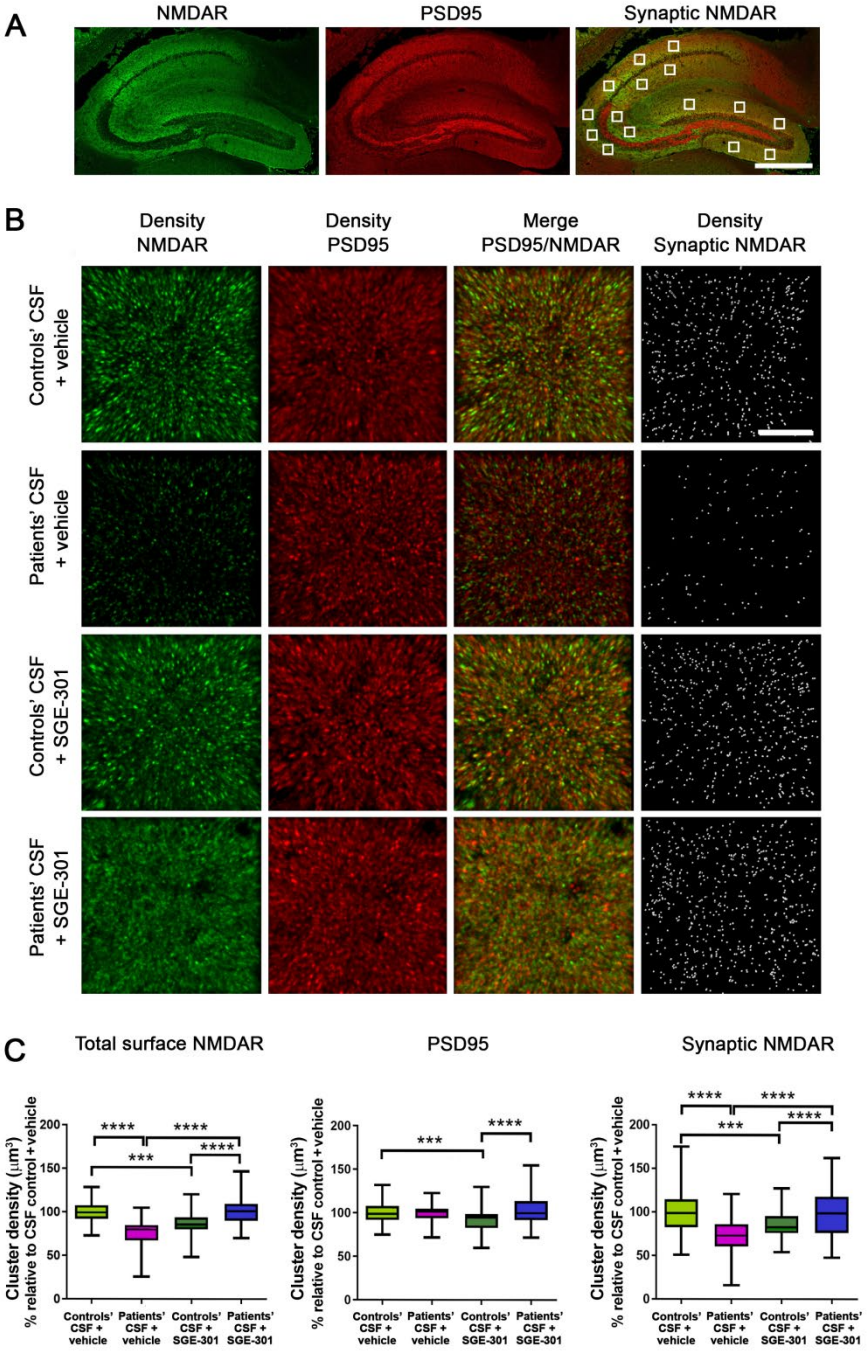

A
NMDAR
PSD95
Synaptic NMDAR
B
Density NMDAR
Density PSD95
Merge PSD95/NMDAR
Density Synaptic NMDAR
Controls' CSF + vehicle
Patients' CSF + vehicle
Controls' CSF + SGE-301
Patients' CSF + SGE-301
C
Total surface NMDAR
PSD95
Synaptic NMDAR
Cluster density (µm³) % relative to CSF control+vehicle
Controls' CSF + vehicle
Patients' CSF + vehicle
Controls' CSF + SGE-301
Patients' CSF + SGE-301

FIGURE 4

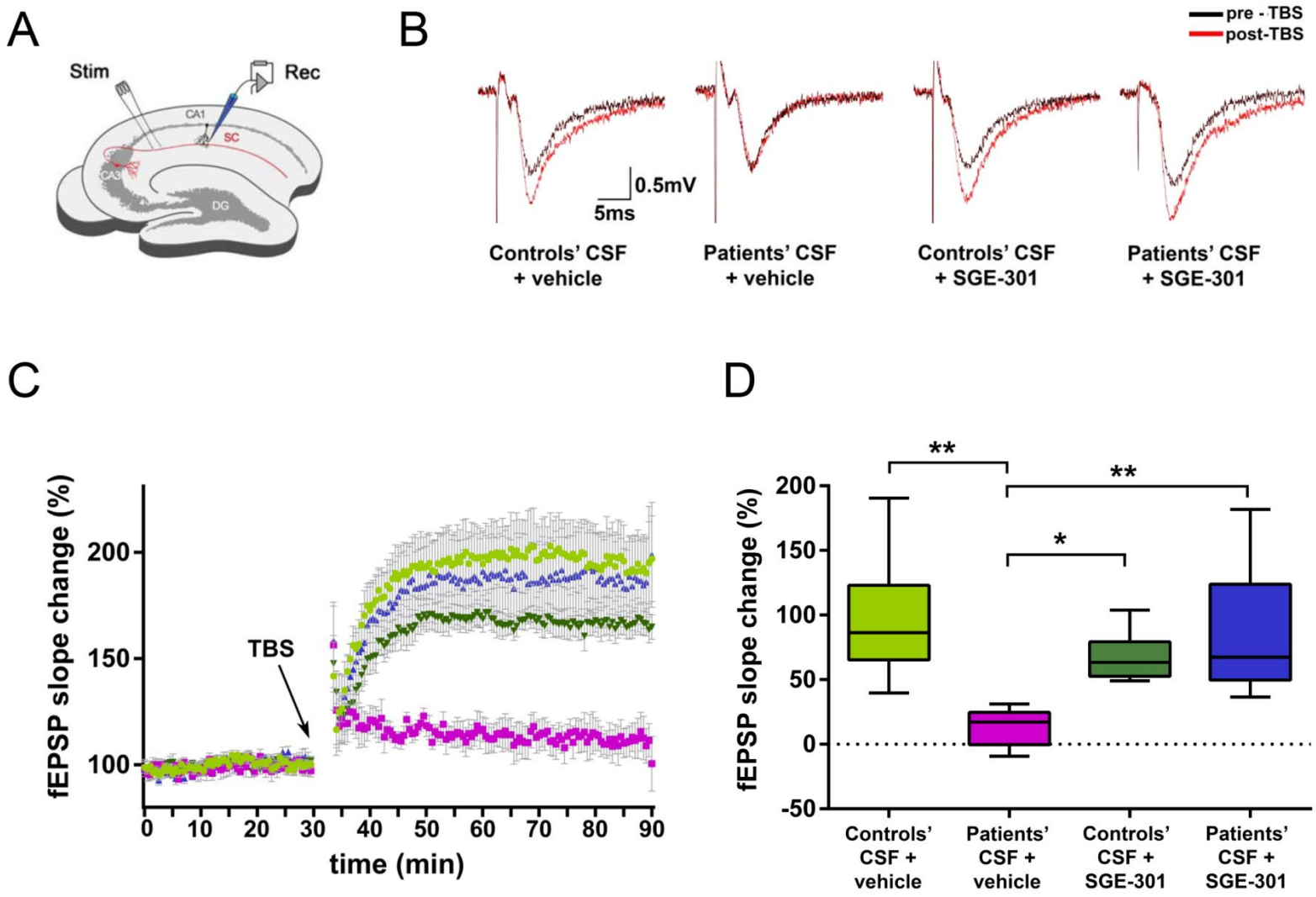
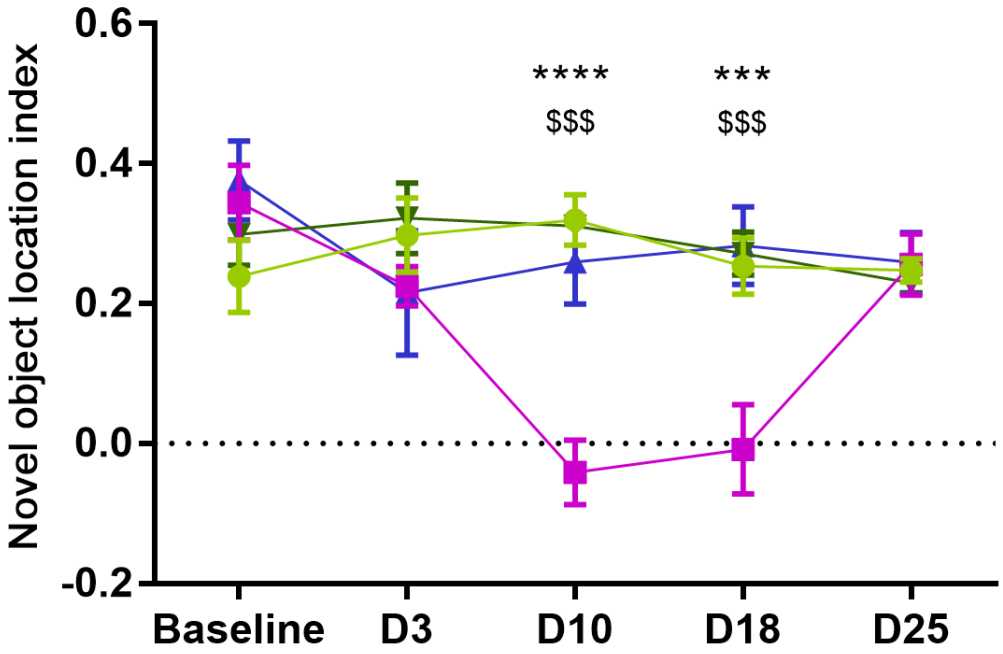
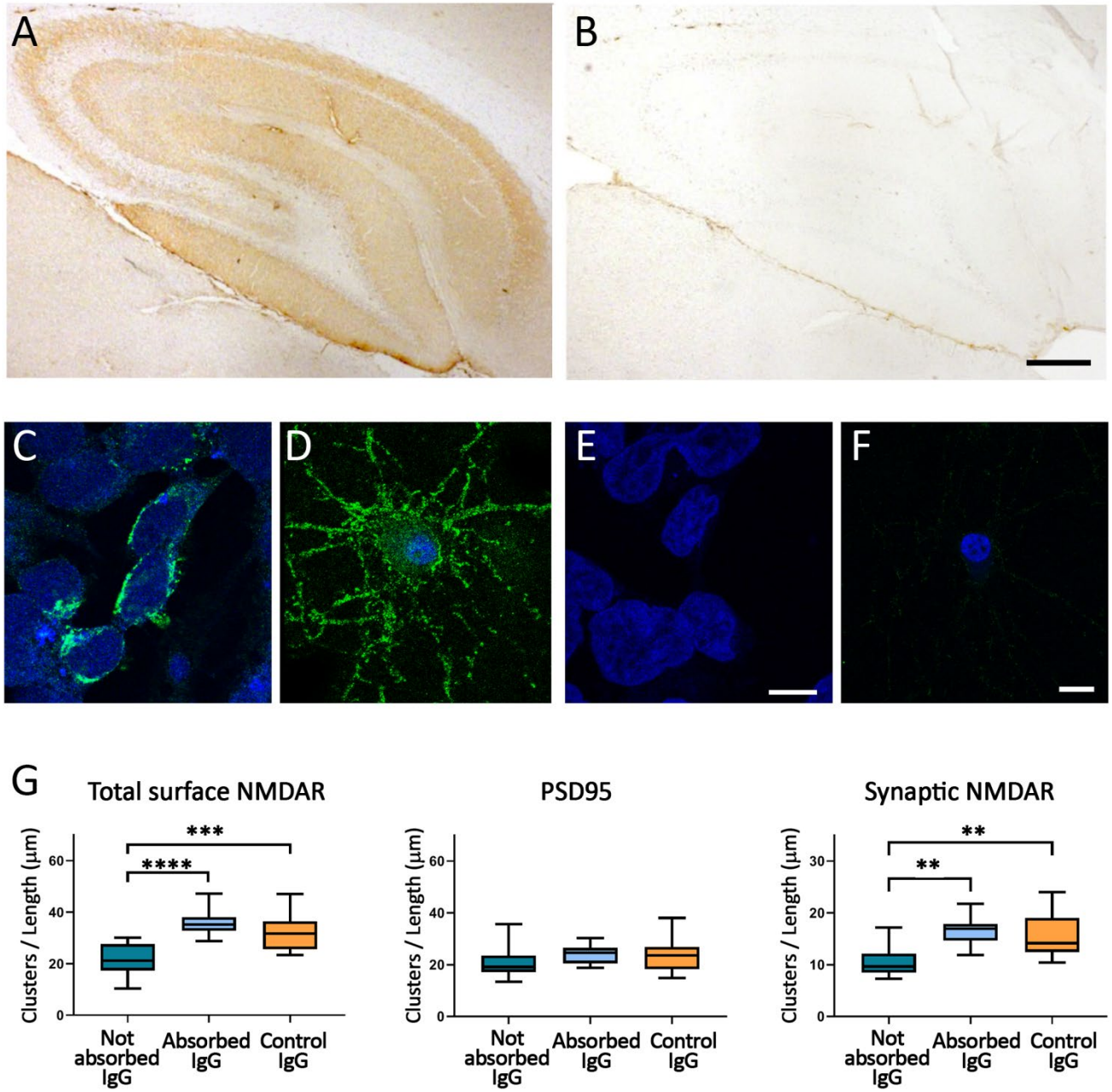

A
Stim
Rec
CA1
SC
CA3
DG
B
pre - TBS
post-TBS
0.5mV
5ms
Controls' CSF + vehicle
Patients' CSF + vehicle
Controls' CSF + SGE-301
Patients' CSF + SGE-301
C
fEPSP slope change (%)
200
150
100
TBS
0
10
20
30
40
50
60
70
80
90
time (min)
D
fEPSP slope change (%)
200
150
100
50
0
-50
**
**
*
Controls' CSF + vehicle
Patients' CSF + vehicle
Controls' CSF + SGE-301
Patients' CSF + SGE-301

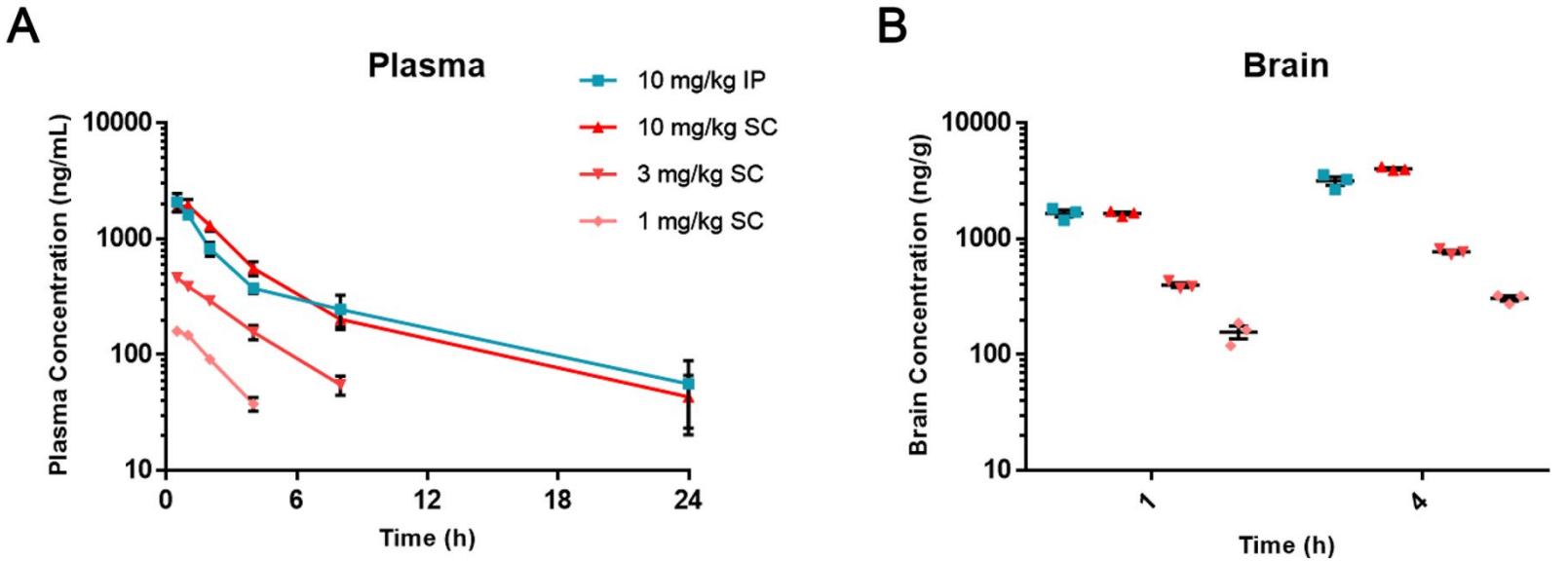
FIGURE 5


Novel object location index
0.6
0.4
0.2
0.0
-0.2
****
$$$
***
$$$
Baseline
D3
D10
D18
D25

SUPPL FIGURE 1



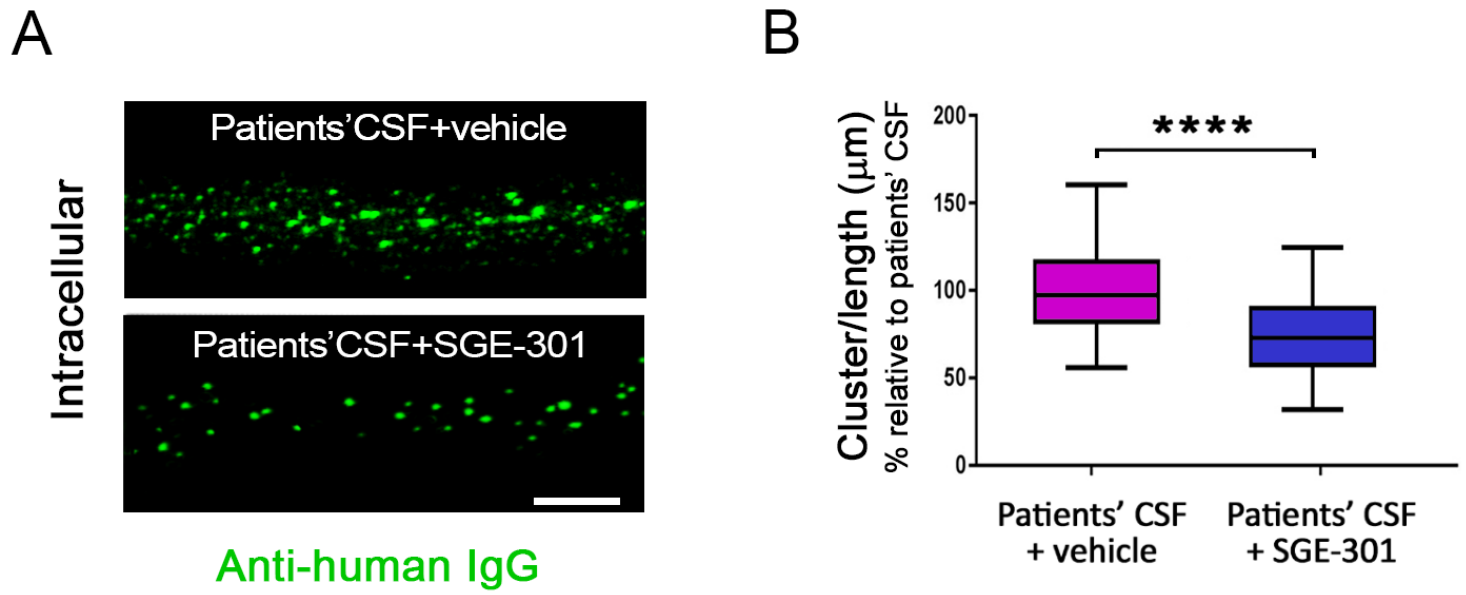
SUPPL FIGURE 2



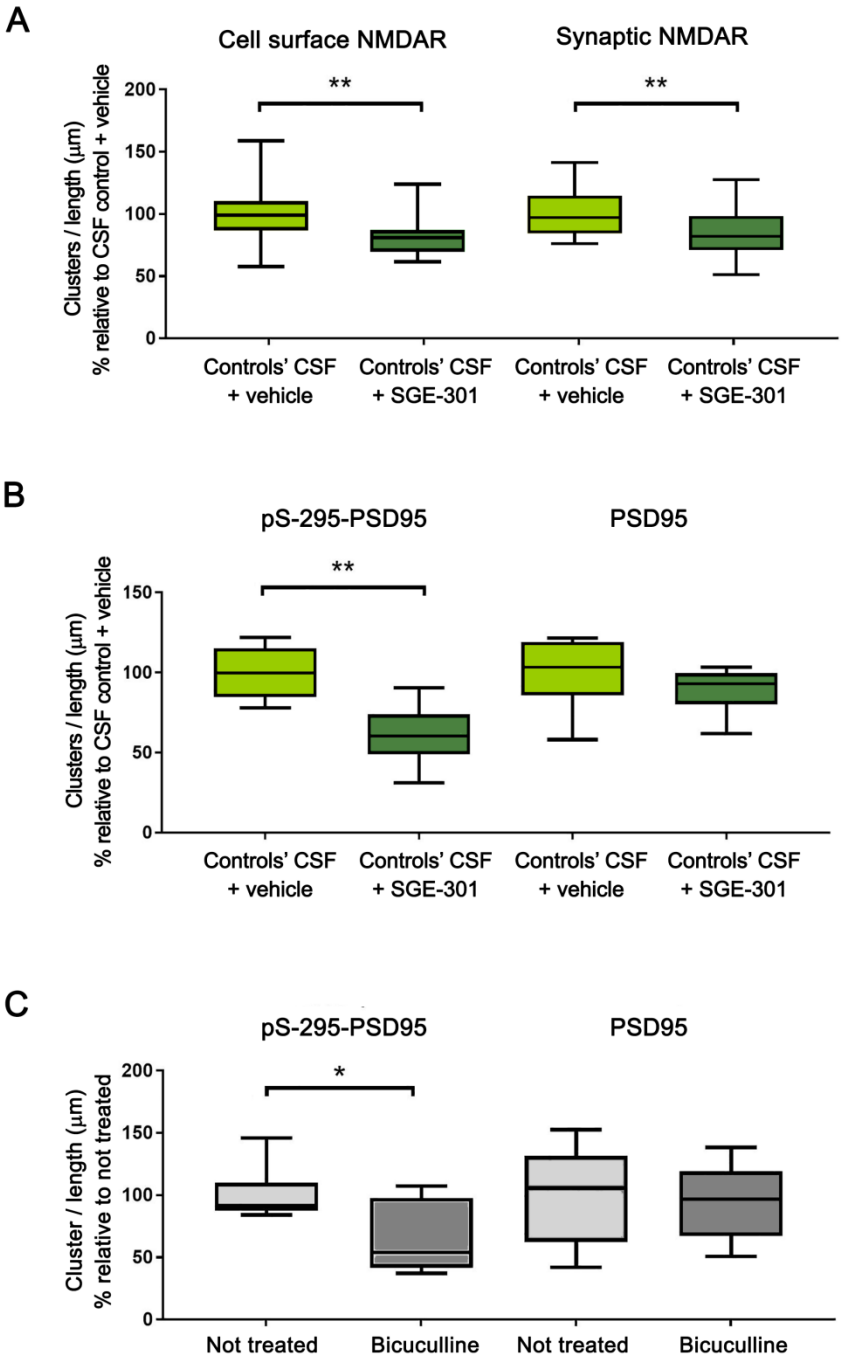
C

		10 mg/kg IP	10 mg/kg SC	3 mg/kg SC	1 mg/kg SC
C_{max}	ng/mL	2190	2083	462	164
T_{max}	h	0.67	0.83	0.50	0.67
$T_{1/2}$	h	5.4	4.3	2.5	1.5
AUC_{last}	ng*h/mL	6487	7340	1491	355

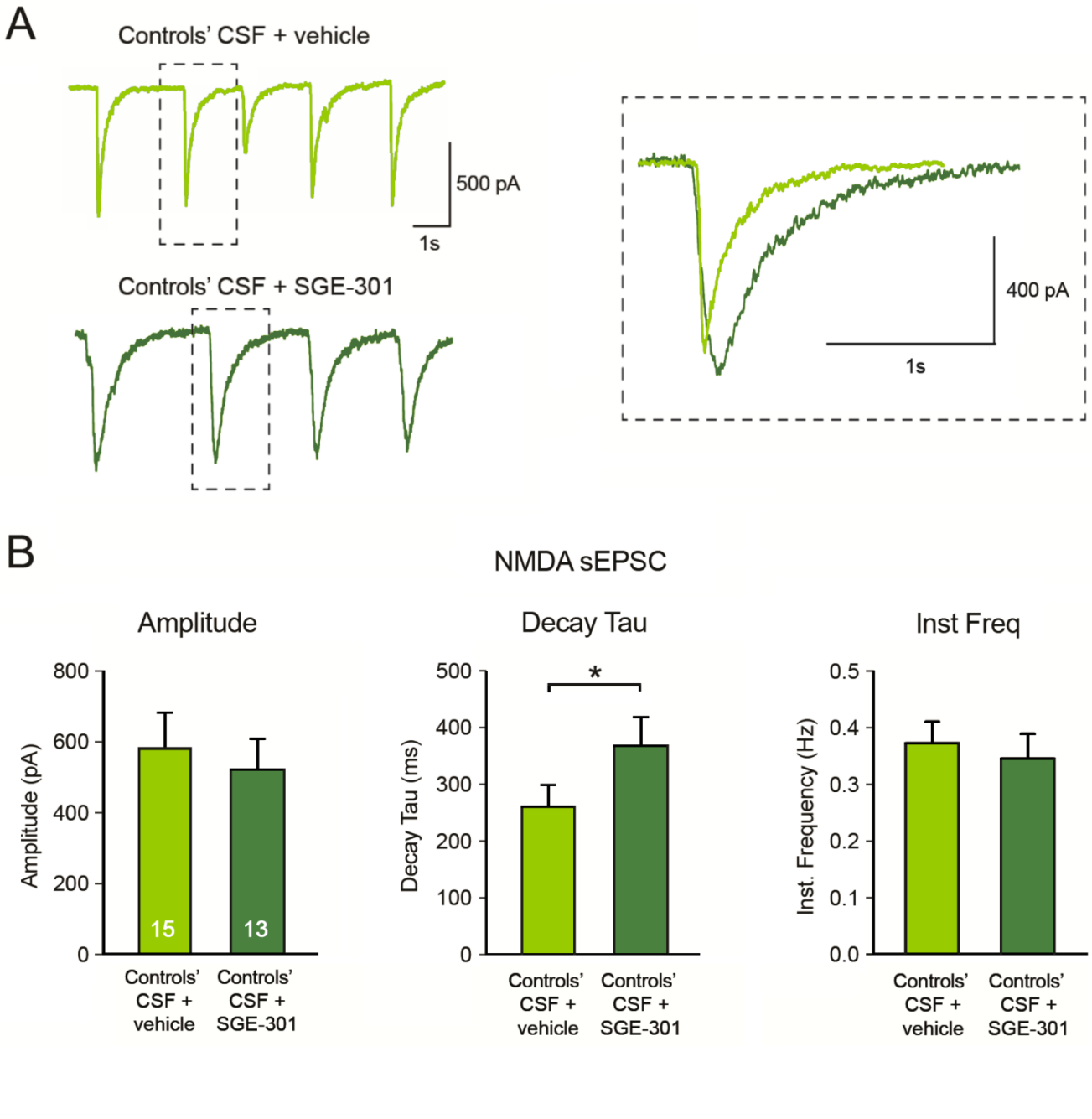
SUPPL FIGURE 3



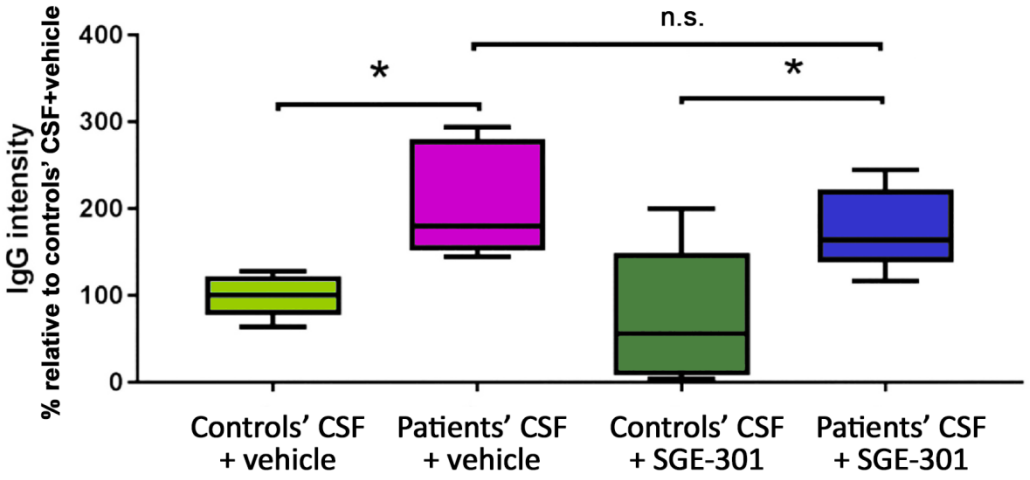
SUPPL FIGURE 4



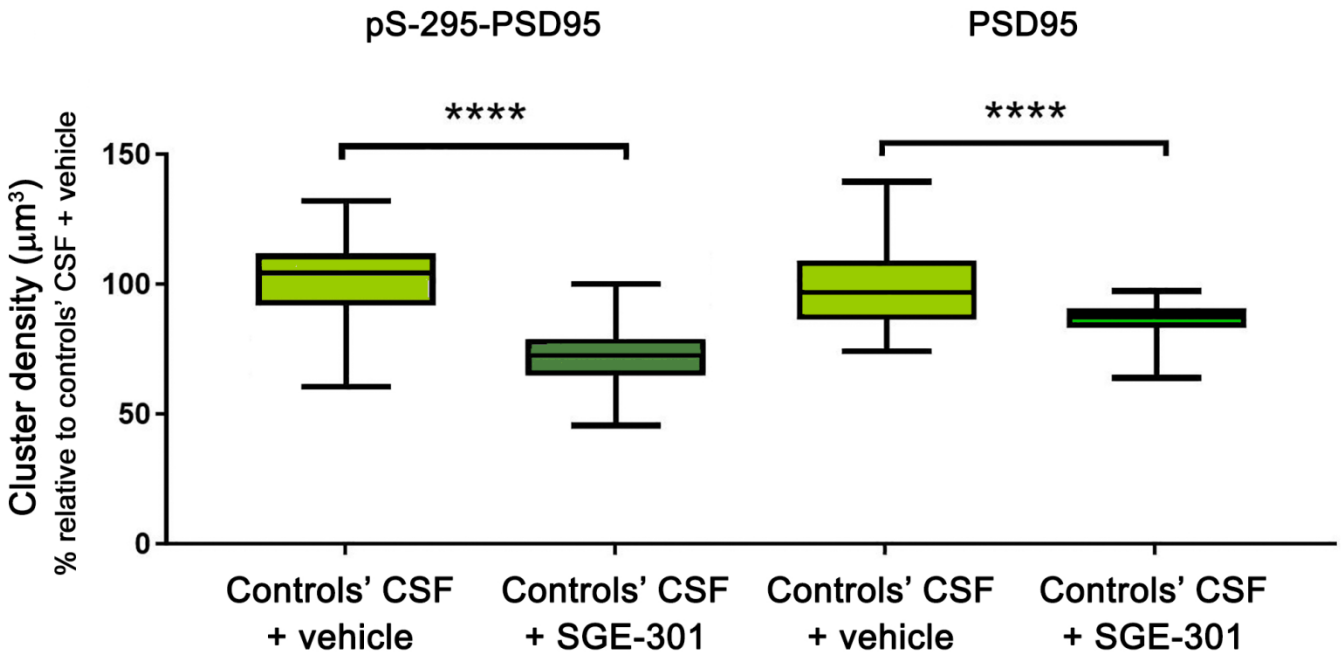
SUPPL FIGURE 5



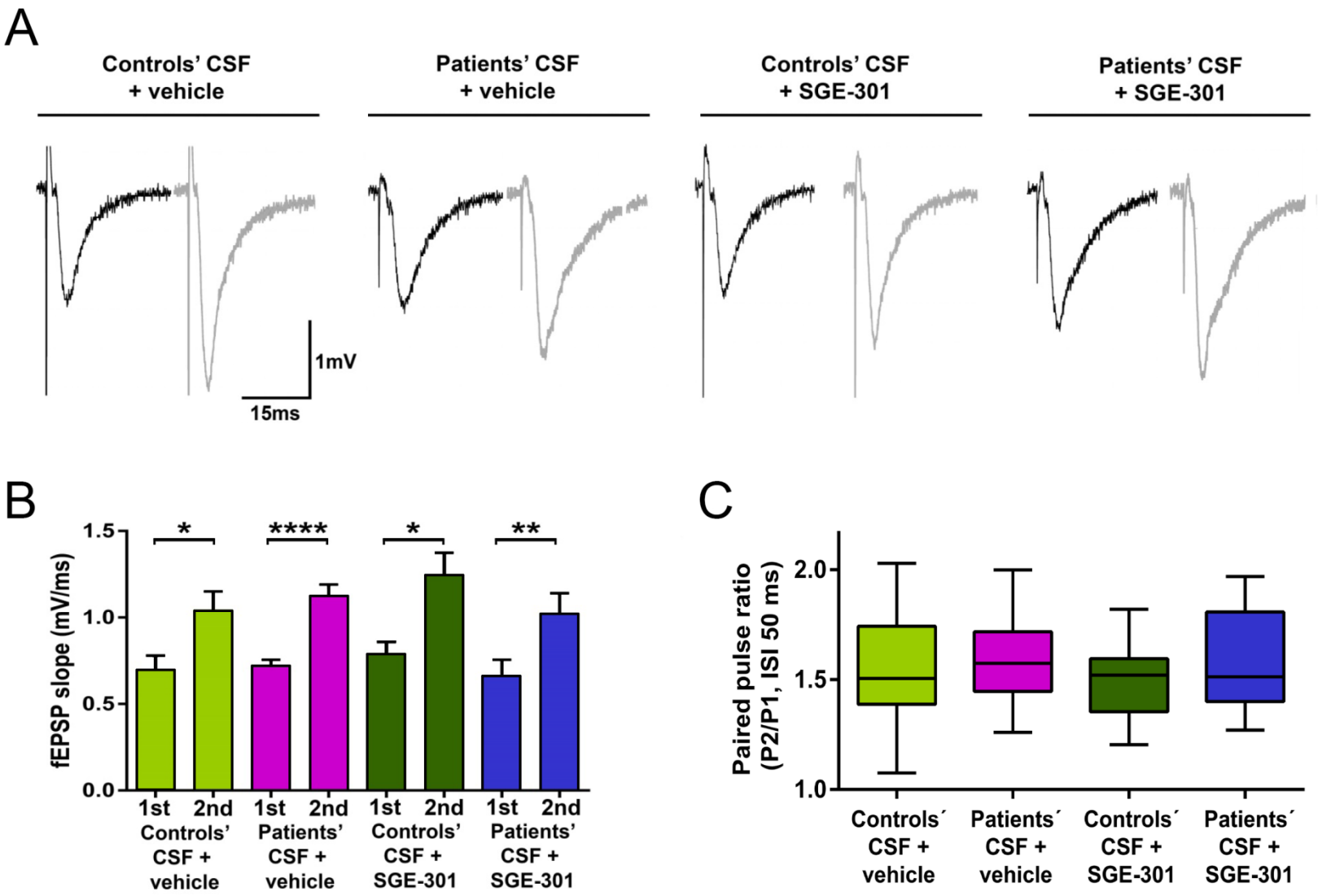
SUPPL FIGURE 6



SUPPL FIGURE 7



SUPPL FIGURE 8



SUPPL FIGURE 9