Supplemental Table 1: Summary of patients whose CSF or IgG were used in all studies

Gender, age	Tumor[a]	CSF[b]	Brain MRI	CSF NR1 antibody titer (rfu)[c]	Treatment	Outcome[d]
F, 35	Ovarian teratoma (M)	67 WBC	Normal	3411488	Tumor removal; C, IVIg	Partial improvement
F, 28	Bilateral ovarian teratoma (M)	60 WBC	Normal	2963488	Tumor removal; RTX, CTX	Full recovery
F, 13	Ovarian teratoma (Im)	46 WBC	Normal	1257440	Tumor removal; PLEX, IVIg	Full recovery
F, 22	Ovarian teratoma (M)	20 WBC, OB	Normal	532239	Tumor removal; C, IVIg, PLEX	Partial improvement
F, 35	Ovarian teratoma (M) (autopsy)	189 WBC	Increased FLAIR signal in medial temporal lobes	376408	C, IVIg, PLEX, CTX	Died[e]
F, 18	Ovarian teratoma (Im)	88 WBC, OB	Mild transient leptomeningeal enhancement	335792	Tumor removal; PLEX, C	Full recovery
F, 24	No tumor	49 WBC, OB	Normal	326550	IVIg	Partial improvement
F, 17	Ovarian teratoma (M)	480 WBC	Increased FLAIR signal in medial temporal lobes	285936	Tumor removal; C, IVIg, RTX	Partial improvement
F, 12	No tumor	Normal	Normal	103336	C, IVIg	Partial improvement
F, 25	Ovarian teratoma (Im)	14 WBC	Normal	95207	Tumor removal; C, IVIg, PLEX	Full recovery
F, 38	Ovarian teratoma (M)	Normal	Normal	61744	Tumor removal; PLEX	Full recovery
F, 18	Bilateral ovarian teratoma (M)	57 WBC	Normal	26088	Tumor removal; C, PLEX	Full recovery
F, 24	Ovarian teratoma (M) (autopsy)	219, OB	Increased FLAIR signal in parietal cortex; mild leptomeningeal enhancement	1843616	Supportive care	Died[f]

(a) All tumors contained nervous tissue; 5/5 tumors examined had expression of NMDARs (Dalmau et al., 2007).

(b) Normal: $\leq$ 4/ microliter.

(c) rfu: reference fluorescence units. Values in 100 randomly selected negative control CSF: <5000 rfu.

[d] Full recovery: able to return to all activities; Partial recovery: patient living at home, independent for most daily activities, but unable to return to work at the time of this report.

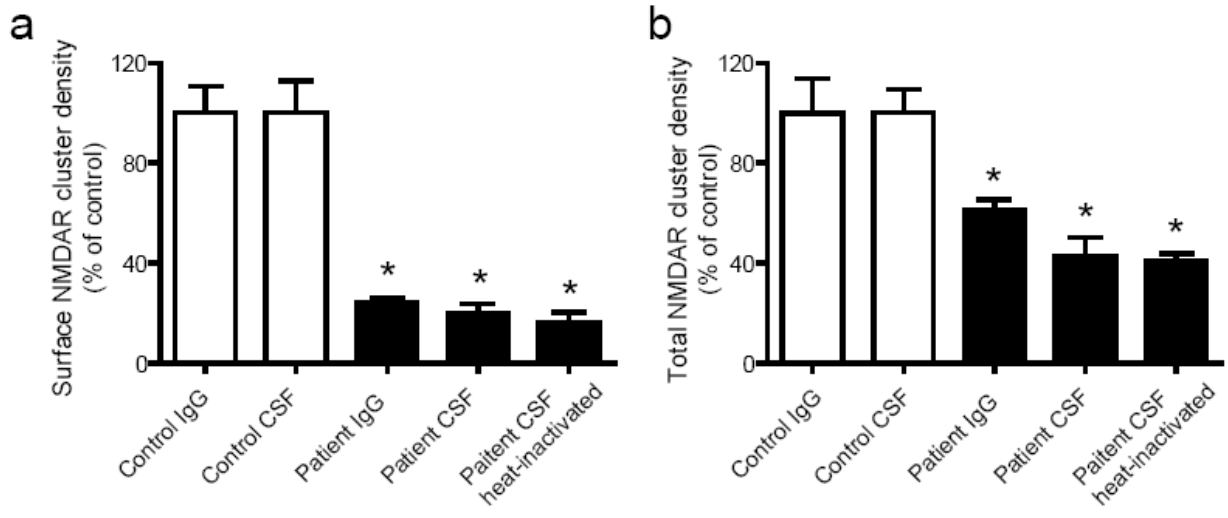
[e] Hippocampal sections from this patient were used for anti-NR1 immunostaining; see Fig. 5.

[f] Hippocampal sections from this patient were used for anti-NRI immunostaining; see Fig. 5; the limited amount of CSF and/or IgG from this patient precluded their use in other experiments.

CSF from the first 12 patients was used in the experiments in Fig. 1a, b. IgG from 10 of these patients was used in the experiments in Fig. 1c, d and Supplemental Fig. 2. Individual patient CSF and/or IgG were used for all other experiments.

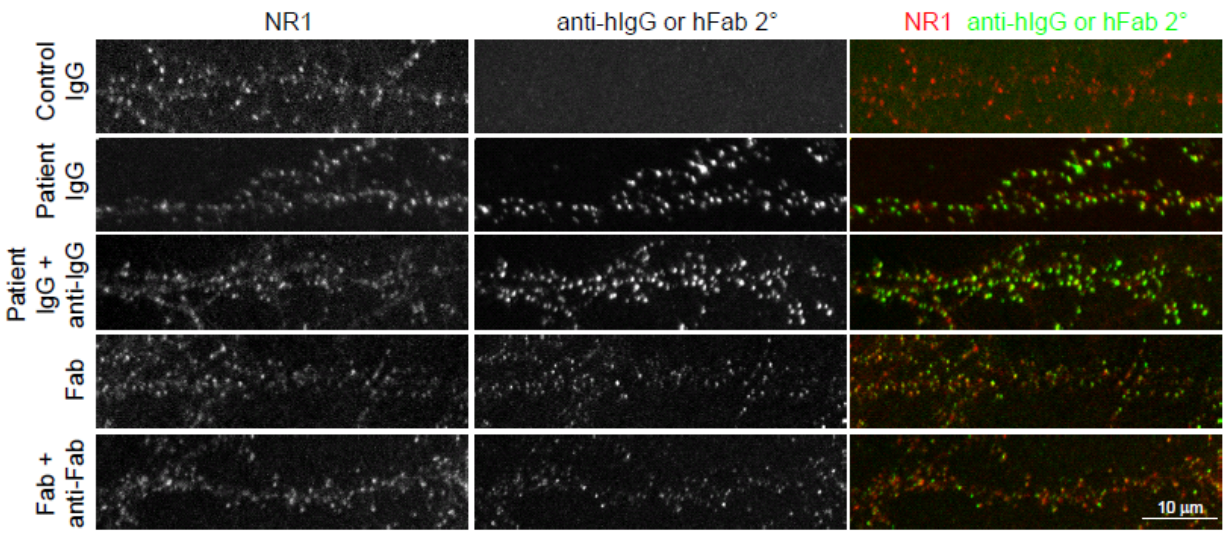
Abbreviations: M, mature; Im, immature; WBC, white blood cells; OB, oligoclonal bands; n.a., not available; C, corticosteroids; RTX, Rituximab; PLEX, plasma exchange; CTX, Cyclophosphamide; IVIg, intravenous immunoglobulin; FLAIR: fluid-attenuated inversion recovery.

Supplemental Figures



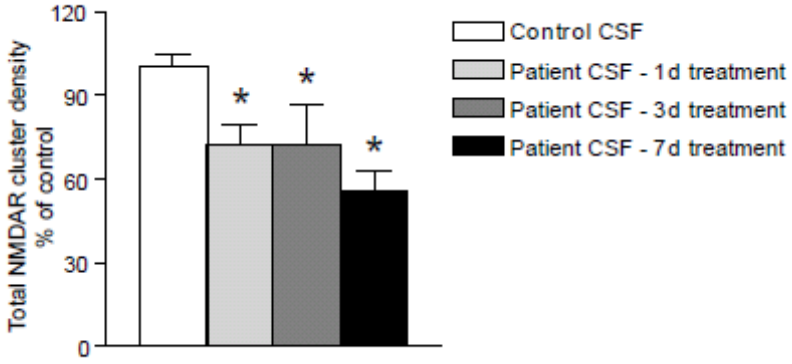
Supplemental Figure 1: Patient IgG and CSF treatment have similar effects and these effects are not mediated via the complement pathway

(a) Quantification of hippocampal neurons immunostained for surface NMDAR clusters treated with Control IgG, CSF, patient IgG, CSF, and heat inactivated patient CSF. Treatment with patient IgG and CSF for one day decrease surface NMDARs to a similar extent. Heat inactivated patient CSF also decrease surface NMDARs to a similar extent as patient IgG and CSF, suggesting that these effects are not mediated by complement-mediated pathways (n = 18 cells, 3 independent expts.; 1 patient, 1 control sample; One-way ANOVA test followed by Bonferroni's multiple comparison test, $p < 0.001$). **(b)** Quantification of dissociated hippocampal neurons immunostained for total NMDARs treated with Control IgG, CSF, patient IgG, CSF, and heat inactivated patient CSF.



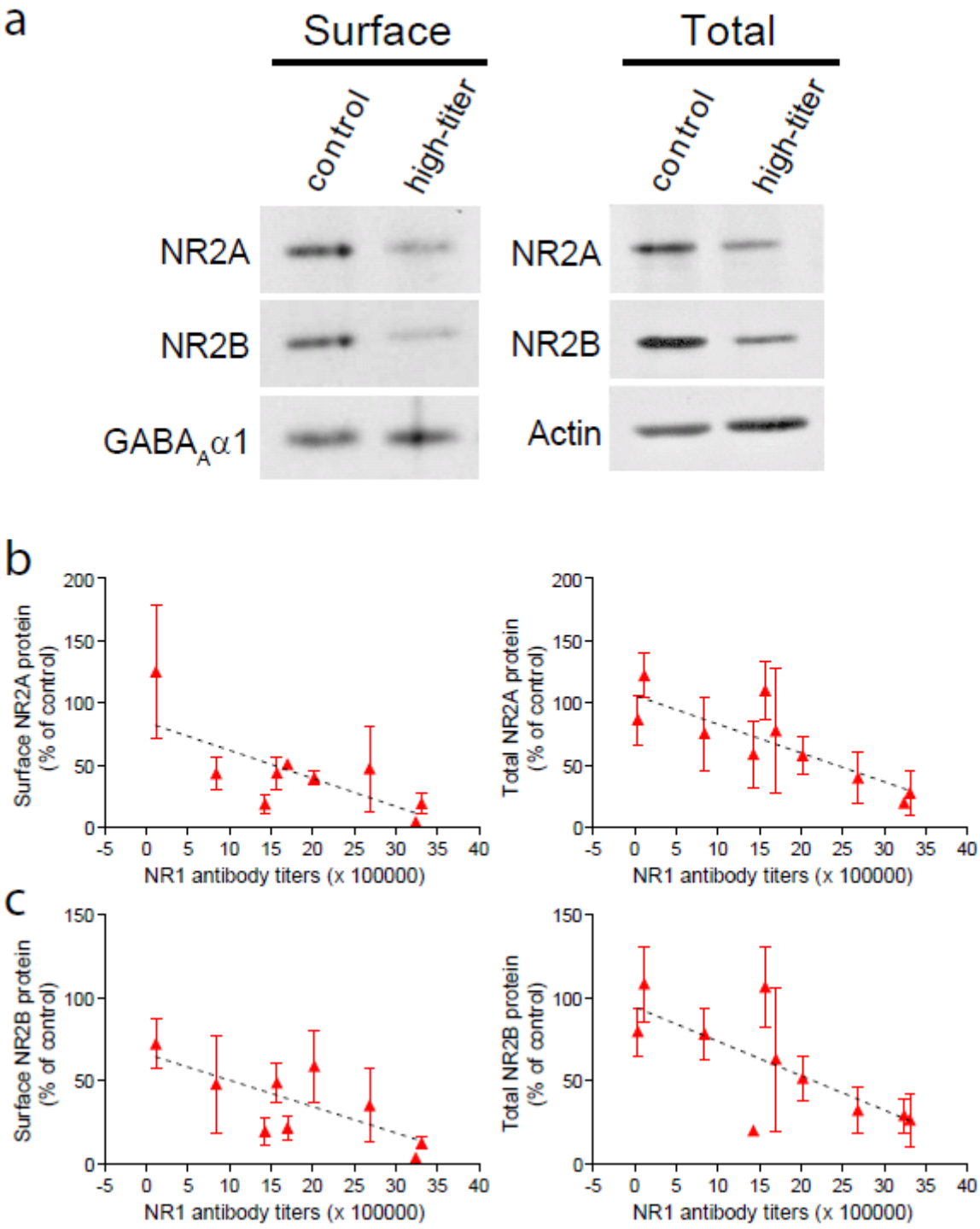
Supplemental Figure 2: Patient antibody Fab fragments colocalize with NMDA receptor clusters

Hippocampal neurons immunostained for NMDAR clusters in neurons treated for 1 day with control IgG (top row), patient IgG (middle top), patient IgG clustered with anti-IgG secondary antibodies (middle), patient Fab fragments (middle bottom), patient Fab fragments reclustered with anti-Fab secondary antibodies (bottom). Color overlays of NR1 (red) and human IgG (green) are shown at right. While patient IgG and patient IgG + anti-IgG stain neurons more intensely, patient Fab fragments and patient Fab fragments + anti-Fab colocalize with NMDARs to a similar extent (n = 18 cells, 3 independent expts.; 1 patient, 1 control sample). Scale bar = 10 µm. Abbreviations: anti-hIgG 2°, anti-human IgG secondary antibody; anti-hFab 2°, anti-human Fab secondary antibody.



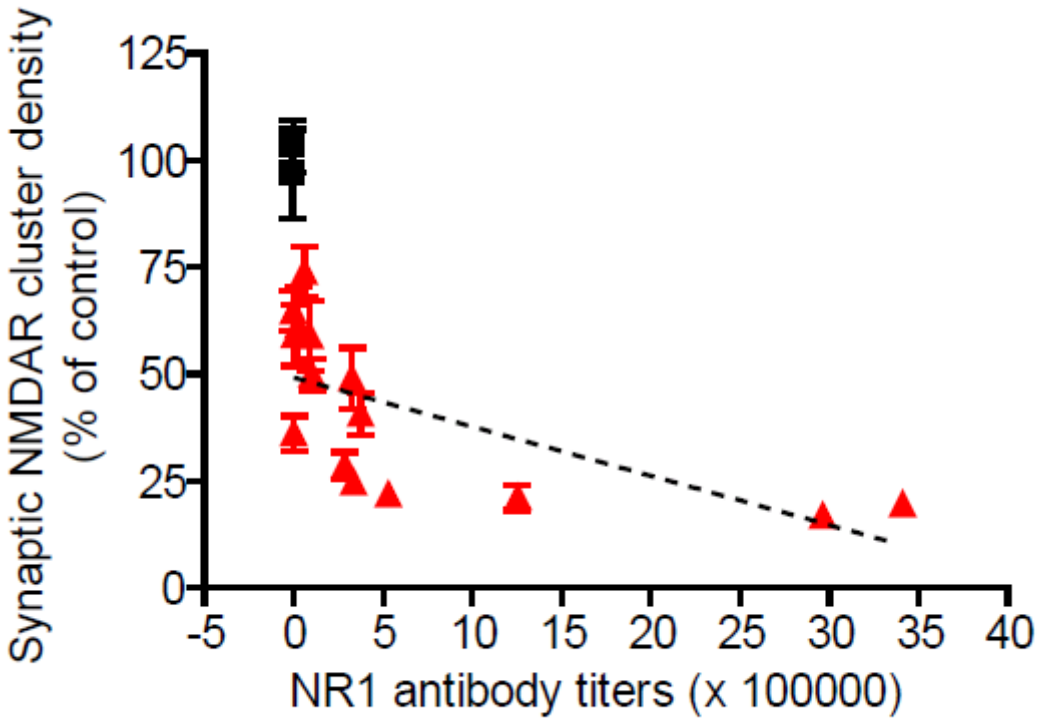
Supplemental Figure 3: Treatment with Patient CSF for 1, 3, or 7 days decreases total NMDAR cluster density.

(a) Total NMDAR cluster density after treatment with CSF for 1, 3, or 7 days, each of which decreased NMDAR cluster density to a similar extent. All values are mean ± s.e.m. (n = 18 cells, 3 independent expts.; 1 patient, 1 control sample, One-way ANOVA test followed by Bonferroni's multiple comparison test, $p < 0.05$).



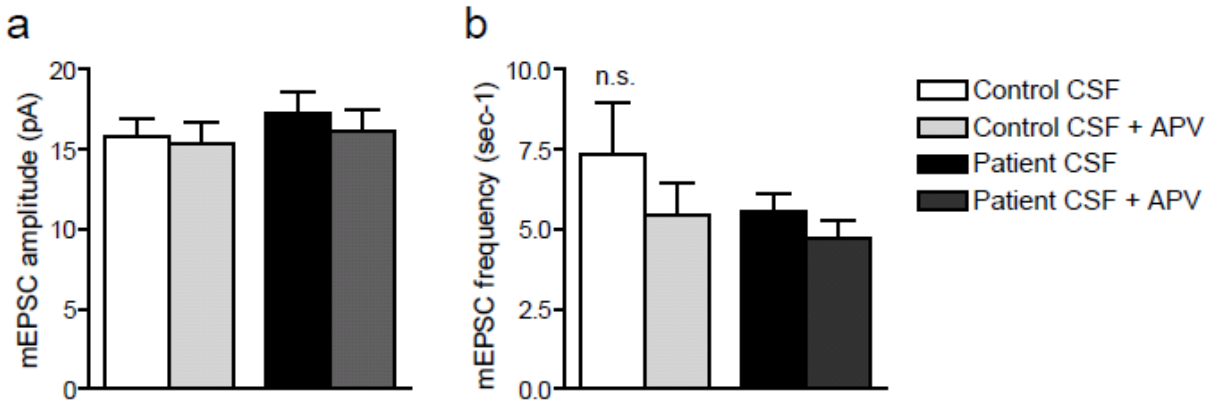
Supplemental Figure 4: Patient IgG treatment decreases surface and protein of NMDA receptor NR2A/B subunits in a titer dependent fashion

(a) Western blots of surface and total NMDAR, NR2A and NR2B protein. Treatment with patient IgG reduced surface as well as total NMDAR, NR2A and NR2B protein. $GABA_A\alpha1$ and actin are loading controls for surface and total protein, respectively. This blot was reprobed with antibodies against NR1 and displayed in Fig 1b and is representative of the data set. **(b)** Quantification of surface (left) and total (right) NMDAR NR2A protein after treatment with IgG from several patients with different antibody titer. IgG from patients with higher titer resulted in a greater decrease in surface and total NMDAR NR2A protein than patients with a lower titer. Thus treatment with patients' CSF for 1 day results in a titer-dependent decrease in NR2A protein (linear regression analysis; surface $R^2 = 0.35$, $p < 0.005$; lysate $R^2 = 0.33$, $p < 0.002$). **(c)** Quantification of surface (left) and total (right) NMDAR NR2B protein after treatment with IgG from several patients with different antibody titer. IgG from patients with higher titer resulted in a greater decrease in surface and total NMDAR NR2B protein than patients with a lower titer. Thus treatment with patients' CSF for 1 day results in a titer-dependent decrease in NR2B protein (linear regression analysis; surface $R^2 = 0.23$, $p < 0.02$; lysate $R^2 = 0.29$, $p < 0.002$). All values are shown as mean ± s.e.m. (n = 3-5 Western blots, 10 patient, 2 control samples, from independent expts. with individual patients' IgG (Supplemental Table 1)).



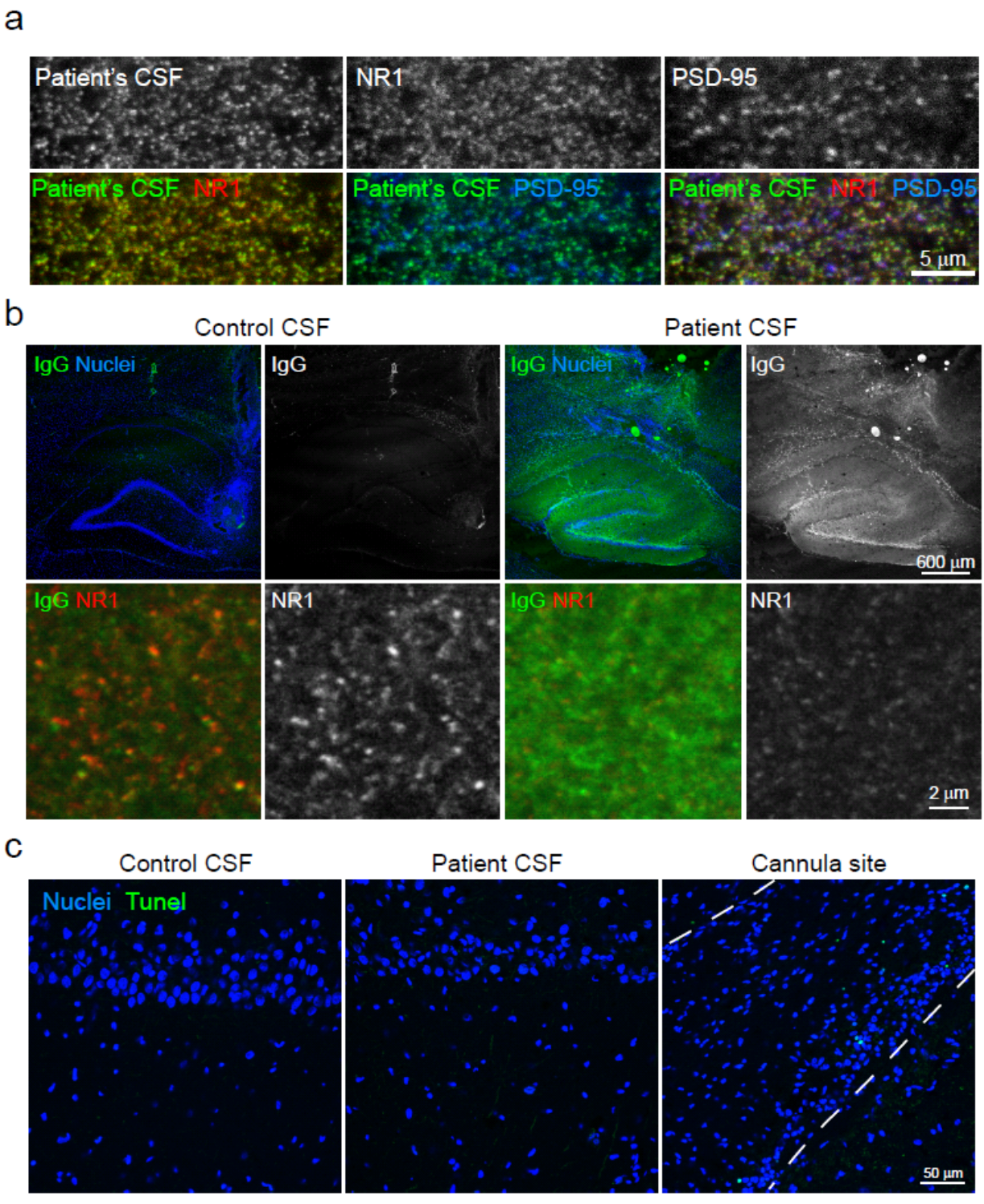
Supplemental Figure 5: Patient IgG treatment decreases synaptic localization of NMDA receptors in a titer dependent fashion

Synaptic NMDAR cluster density after treatment with CSF from several patients with different antibody titer for 1 day, showing a titer-dependent decrease in synaptic NMDAR cluster density Synaptic NMDARs are defined as the colocalization between commercial NMDAR staining (intracellular NR1 epitope) and presynaptic marker Bassoon (linear regression analysis; R^2 = 0.43, $p < 0.008$). This data was obtained from the data set displayed in Fig. 1. See also Figure 2.



Supplemental Figure 6: Patient CSF treatment does not affect mEPSC frequency or amplitude

(a) Quantification of mEPSC amplitude in neurons treated with control CSF, patient CSF, with and without APV. The total mEPSC amplitude (which represents the amount of functional postsynaptic AMPA receptors) is not significantly different among control, patient CSF or APV conditions. All values are shown as mean ± s.e.m. (n = 13 cells, 7 control CSF, 6 patient CSF, 4 independent expts.; 1 patient, 1 control sample; pairwise comparison, Student's t test, $p > 0.4$). **(b)** Quantification of mEPSC frequency in neurons treated with and without control, patient CSF, and APV. The frequency of mEPSCs (which represents the number of excitatory synapses) is not significantly different between control, patient CSF or APV though the trend of lower frequency of patient CSF and APV treated conditions could be the result of blockade of silent synapses which have been shown to contribute to mEPSC frequency(Liao et al., 1995; Liao et al., 2001). All values are shown as mean ± s.e.m. (n = 6 cells, 4 independent expts.; 1 patient, 1 control sample; pairwise comparison, Student's t test, $p > 0.2$).


a
Patient's CSF
NR1
PSD-95
Patient's CSF NR1
Patient's CSF PSD-95
Patient's CSF NR1 PSD-95
5 µm
b
Control CSF
Patient CSF
IgG Nuclei
IgG
IgG Nuclei
IgG
600 µm
IgG NR1
NR1
IgG NR1
NR1
2 µm
c
Control CSF
Patient CSF
Cannula site
Nuclei Tunel
50 µm

Supplemental Figure 7: Patient CSF recognizes NMDA receptor clusters *in vivo* and infusion into rat hippocampus results in deposition of human IgG without increasing cell death

(a) Rat brain sections immunostained with patient CSF (top left), NMDARs (top middle), and a postsynaptic protein, PSD-95 (top right). Clusters immunostained with patient CSF are highly colocalized with NMDARs (yellow puncta, bottom left). Clusters immunostained with patient CSF colocalize with PSD-95 to a similar extent as NMDARs (compare bottom middle to bottom right). (n = 9 images, 3 independent expts.; 1 patient, 1 control sample).**(b)** Brain sections from rats infused with control (left) or patient CSF (right) into one hippocampus, and immunostained with human IgG, NR1, and TO-PRO to label nuclei. The deposition of human IgG was seen in the hippocampus of rats infused with patient CSF but not control CSF**.** Below, higher magnification views of the CA1 region of the hippocampus show that areas with human IgG deposits have reduced NMDAR clusters (see Fig. 5) and decreased overall staining intensity (n=6 animals; 3 infused with patient CSF, 3 with control samples). **(c)** Brain sections from rats infused with control or patient CSF and immunostained with TO-PRO to label nuclei and TUNEL to label apoptotic cells. Infusion with control (left) or patient CSF (middle) did not cause significant cell death. While several apoptotic cells were found along the cannula tract (right), the total number and distribution did not differ between rats infused with control or patient CSF.

Supplemental References

Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, Baehring JM, Shimazaki H, Koide R, King D, Mason W, Sansing LH, Dichter MA, Rosenfeld MR, Lynch DR (2007) Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 61:25-36.

Liao D, Hessler NA, Malinow R (1995) Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. Nature 375:400-404.

Liao D, Scannevin RH, Huganir R (2001) Activation of silent synapses by rapid activity-dependent synaptic recruitment of AMPA receptors. J Neurosci 21:6008-6017.