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Control samples (200 subjects)

Control samples included 21 sera from healthy blood donors, 14 sera from patients with neurodegenerative disorders, 20 sera from patients with multiple sclerosis, 38 sera (and 32 paired CSF) from patients with well-characterized autoimmune encephalitis (18 NMDAR, 8 LGI1, 3 AMPAR, 3 Caspr2, 3 GABAaR, 3 GABAbR), and 107 CSF (with 95 paired sera) from patients with encephalitis suspected to be autoimmune (reactivity against unknown neuropil antigens).

Immunohistochemistry with rat brain

Adult female Wistar rats were sacrificed without perfusion, and the brains removed and fixed by immersion in 4% paraformaldehyde for 1 hour at 4ºC, cryoprotected in 40% sucrose for 24 hours, embedded in freezing compound media, and snap frozen in isopentane chilled with liquid nitrogen. Seven-micrometer-thick tissue sections were then sequentially incubated with 0.3% H_2O_2 for 15 minutes, 5% goat serum for 1 hour, and patient or control serum (1:200) or CSF (1:2) at 4°C overnight. After using a biotinylated secondary antibody against human IgG (diluted 1:2000; BA-3000, Vector laboratories) for 1 hour at room temperature (RT), the reactivity was developed with the avidin-biotin-peroxidase method. Results were photographed with an AxioCam MRc colour camera adapted to a confocal microscope (Zeiss LSM710) and analysed with Zen software (Zen 2012 blue edition 1.1.1.0, Zeiss).

Immunocytochemistry with neuronal cultures

Dissociated cell cultures of rat hippocampal neurons were prepared as reported.[1] Live neurons grown on coverslips were incubated for 1 hour at 37°C with patient or control serum (final dilution 1:200) or CSF (1:5). After removing the media and washing with

phosphate-buffered saline (PBS), neurons were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton X-100, and immunolabeled with Alexa Fluor 488 goat anti-human IgG (1:1000, A11013, Molecular Probes) for 1 hour at RT. Images were captured with an epifluorescence microscope using Zeiss Axiovision software (Zeiss, Thornwood, NY, USA).

Immunocompetition assay

To determine whether patients' antibodies were directed against similar epitopes of neurexin-3α, immunocompetition studies were performed. IgG was isolated from a patient whose serum contained high levels of IgG antibodies against neurexin-3α using protein A and G sepharose beads (20423, Pierce), and subsequently eluted and labelled with biotin (Vector, SP1200), as reported.[2] Then, sections of rat brain were pre-incubated with other patients' or control sera (diluted 1:5) overnight at 4ºC, washed in PBS, incubated with the indicated human biotinylated IgG containing neurexin-3α antibodies (diluted 1:20) for 1 hour at RT, and the reactivity was developed with the avidin-biotin-peroxidase method. Two sera were considered to compete for the same neurexin-3α epitopes when pre-incubation of the tissue with one serum abrogated the reactivity of the other patient's IgG. Pictures were taken under a confocal microscope as described above.

Immunoprecipitation

Live neurons obtained as above, were grown in 100 mm dishes (density 1 x 10^6 neurons/dish), and incubated at 37°C with filtered patient serum (diluted 1:200) for 1 hour. Neurons were then washed with PBS, lysed with buffer (NaCl 150mM, EDTA 1mM, tris(hydroxymethyl)aminomethane [Tris]-HCl 100mM, deoxycholate acid 0.5%, 1% Triton X-100 [Sigma Labs], pH 7.5) containing protease inhibitors (P8340; Sigma Labs), and centrifuged at 16.1 x $10^3 g$ for 20 minutes at 4°C. The supernatant was retained and incubated with protein A/G agarose beads (20423; Pierce) overnight at 4°C, centrifuged, and the pellet containing the beads with patient's antibodies bound to the target cell surface antigen was then washed with PBS, resuspended in Laemmli buffer, boiled for 5 minutes, separated in a 4 to 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the proteins visualized with EZBlue gel staining (G1041; Sigma Labs). Visible protein bands precipitated by patient's serum were excised from the gel and analyzed using mass

spectrometry at the Proteomics Core Facility of the Genomics Institute at the Abramson Cancer Center (University of Pennsylvania).

Immunocytochemistry on HEK293 cells

HEK293 cells were transfected with plasmids containing human neurexin-3α (courtesy of Dr. Südhof) or LRRTM2 (sc-114672, Origene). Cells were grown for 24 hours after transfection before assessment. Transfected cells were fixed in 4% paraformaldehyde, permeabilized with 0.3% Triton X-100, blocked in 1% BSA for 60 minutes, and then incubated overnight at 4ºC with patients' serum (1:40) or CSF (1:5) and depending on the antigen of interest one of the following antibodies, anti-neurexin-3α (rabbit polyclonal diluted 1:2000, ABN96 Millipore) or anti-LRRTM2 (rabbit polyclonal diluted 1:500, ab106627 Abcam), followed by incubation with the corresponding fluorescent secondary antibodies, Alexa Fluor 488 goat anti-human IgG and Alexa Fluor 594 goat anti-rabbit IgG, (all used at 1:1000, A11013 and A11012 respectively, Molecular Probes) for 1h at RT. Results were imaged under Apotome 2 fluorescence microscope (Zeiss), using the Zen black software (Zeiss).

Immunoabsorption with HEK cells expressing neurexin-3α

To determine if patients' serum or CSF samples contained additional antibodies that resulted in the indicated brain immunostaining, CSF (diluted 1:80) from a representative patient was pre-absorbed during 6 hours with HEK293 cells expressing neurexin-3α or non-transfected cells. Every hour the culture media (2 ml) with patient's antibodies was moved to a new 3.5 cm plate containing HEK293 cells expressing or not expressing neurexin-3α (6 sequential incubations at RT in 6 hours; all using fixed and permeabilized HEK293 cells). The reactivity of the pre-absorbed and post-absorbed CSF was then assessed using rat brain immunohistochemistry as above.

Quantitation of neuronal death

Hippocampal cultures were treated with patient's or control IgG as indicated above. At *div* 17 cells were washed with PBS, fixed with 4% paraformaldehyde, permeabilized with

0.3% Triton X-100, and blocked for 60 minutes with 1% BSA. Cells were then incubated overnight at 4°C with MAP2 antibody (mouse monoclonal, diluted 1:2000, M1406, Sigma-Aldrich), washed and incubated for 1 hour at RT with Alexa Fluor 594 goat anti-mouse (A-11005, Molecular probes), washed, and mounted with ProlonGold with DAPI (P36935, Molecular Probes). The number of neurons in 10 fields (20x objective) in 4 independent experiments (total 40 fields) were photographed and counted using the public domain Fiji ImageJ software (http://fiji.sc/Fiji).

Quantitation of the number of dendrites, dendritic complexity, and spine density

17 *div* hippocampal neurons treated with patient's IgG or control IgG were fixed and permeabilized as above and incubated with MAP2 antibodies (to identify dendrites; mouse monoclonal, diluted 1:2000, M1406, Sigma-Aldrich) and β-actin antibodies (to identify spines; mouse monoclonal, diluted 1:2000, A1978, Sigma-Aldrich), followed by the secondary antibody Alexa Fluor 594 goat anti-mouse (A-1100, Molecular Probes). Five fields from 3 independent experiments (total 15 fields) per condition were photographed with a 63x objective and 0.6 zoom and standardized z-stacks including 25 optical images were acquired using sequential scanning, 1024×1024 lateral resolution, and Nyquist optimized z sampling frequency. Images were deconvolved and analyzed using a filament tracer algorithm (Imaris suite 7.6.4, Bitplane). Dendrite length was expressed in μm, the number of dendrites was classified according to dendrite order, and dendrites from each category were expressed as percentage of total. To quantify the number of spines, only secondary and tertiary dendrites longer than 20μm were analyzed. Density of spines was expressed as number of spines/20μm.

Supplemental References

1. Buchhalter JR, Dichter MA. Electrophysiological comparison of pyramidal and stellate nonpyramidal neurons in dissociated cell culture of rat hippocampus. *Brain Res Bull* 1991;26:333-338.

2. Furneaux HM, Rosenblum MK, Dalmau J, Wong E, Woodruff P, Graus F, Posner JB. Selective expression of Purkinje-cell antigens in tumor tissue from patients with paraneoplastic cerebellar degeneration. *New England Journal of Medicine* 1990;322:1844-1851.

Table e-1: Sequences of neuronal protein immunoprecipitates

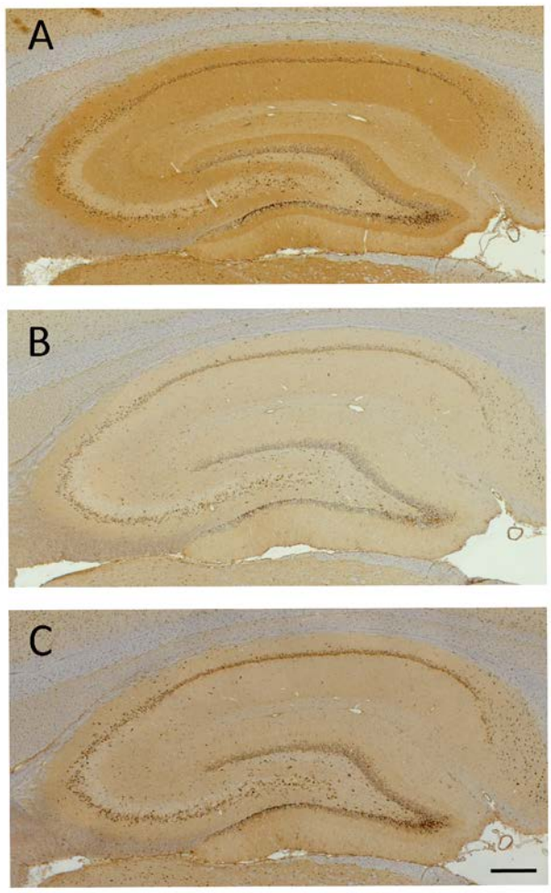
Serum 1:

Peptide sequence	Neurexin 3α peptide identification probability	Sequest X Corr	Sequest delta Cn
EASILSYDGSMYMK	95%	4,70	0,11
FICDCTGTGYWGR	95%	4,21	0,49
AYGLLVATTSR	95%	4,20	0,54
LEFHNIETGIMTEK	95%	3,88	0,33
MGSISFDFR	95%	3,53	0,46
TPVNDGKYHVVR	95%	3,53	0,43
SGGLILYTWPANDRPSTR	95%	3,33	0,37
NGDIDYCELK	95%	3,23	0,36
LPDLINDALHR	95%	3,16	0,36
LMVNLDCIR	95%	3,16	0,36
QLAEMQNAAGVK	95%	3,14	0,36
GPETLYAGQK	95%	3,09	0,39
NGLILHTGK	95%	2,99	0,25
VVTQVINGAK	95%	2,91	0,33
IYGEVVFK	95%	2,65	0,36
VIMPMVMHTEAEDVSFR	95%	2,46	0,40
EENVATFR	95%	2,14	0,30

Serum 2:

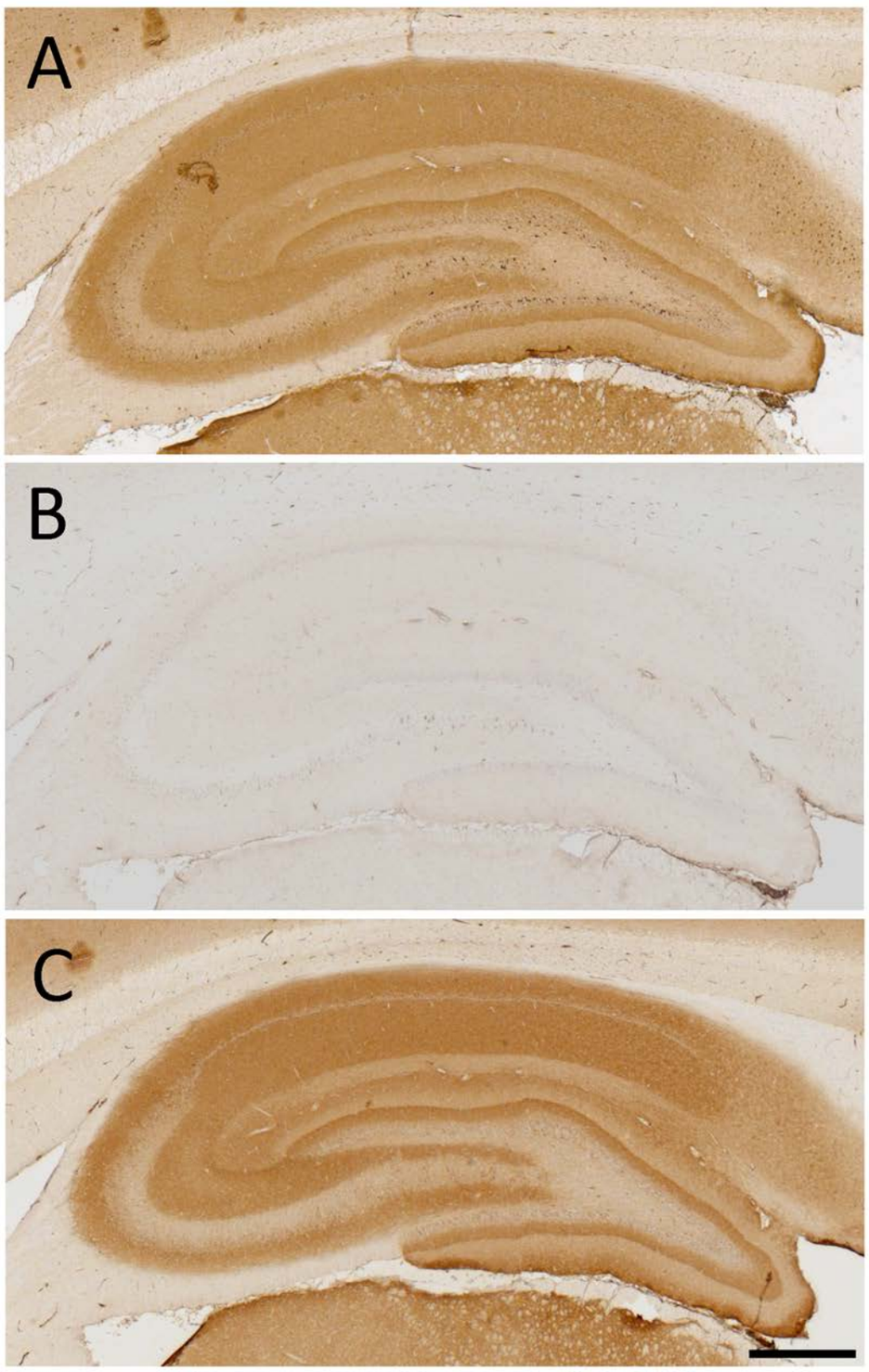
Peptide sequence	Neurexin 3α peptide identification probability	Sequest X Corr	Sequest delta Cn
AYGLLVATTSR	95%	4,42	0,53
TPVNDGKYHVVR	95%	3,48	0,47
VVTQVINGAK	95%	3,15	0,37
QLAEMQNAAGVK	95%	2,42	0,36
NGLIHTGK	95%	3,03	0,48
MGSISFDFR	95%	3,13	0,49
LMVNLDCIR	95%	2,93	0,27
GPETLYAGQK	95%	2,91	0,37

Figure e-1: Patients' antibodies compete for the same antigen



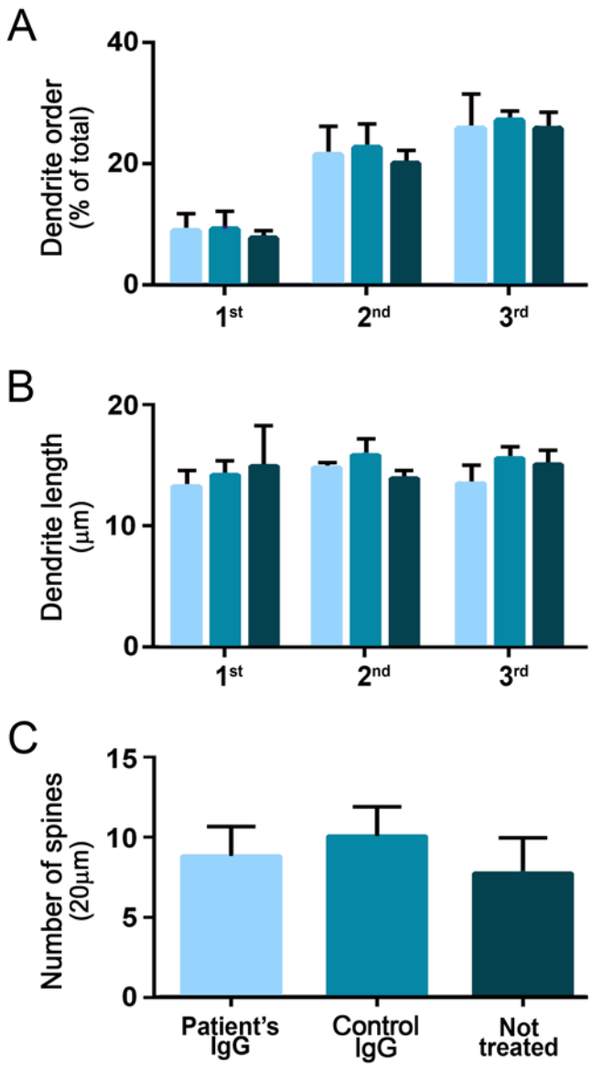
Reactivity of patient's biotinylated IgG with rat hippocampus pre-incubated with serum from a healthy subject (A), serum from the same patient whose IgG has been biotinylated (B), and serum from another patient with neurexin-3α antibodies (C). Note the decrease of reactivity in panels B and C, indicating that the two patients have antibodies that compete for the same neurexin-3α epitopes. Scale bar = 200µm

Figure e-2: Specific absorption of patients' antibodies with HEK293 cells expressing neurexin-3α abrogates the reactivity with brain



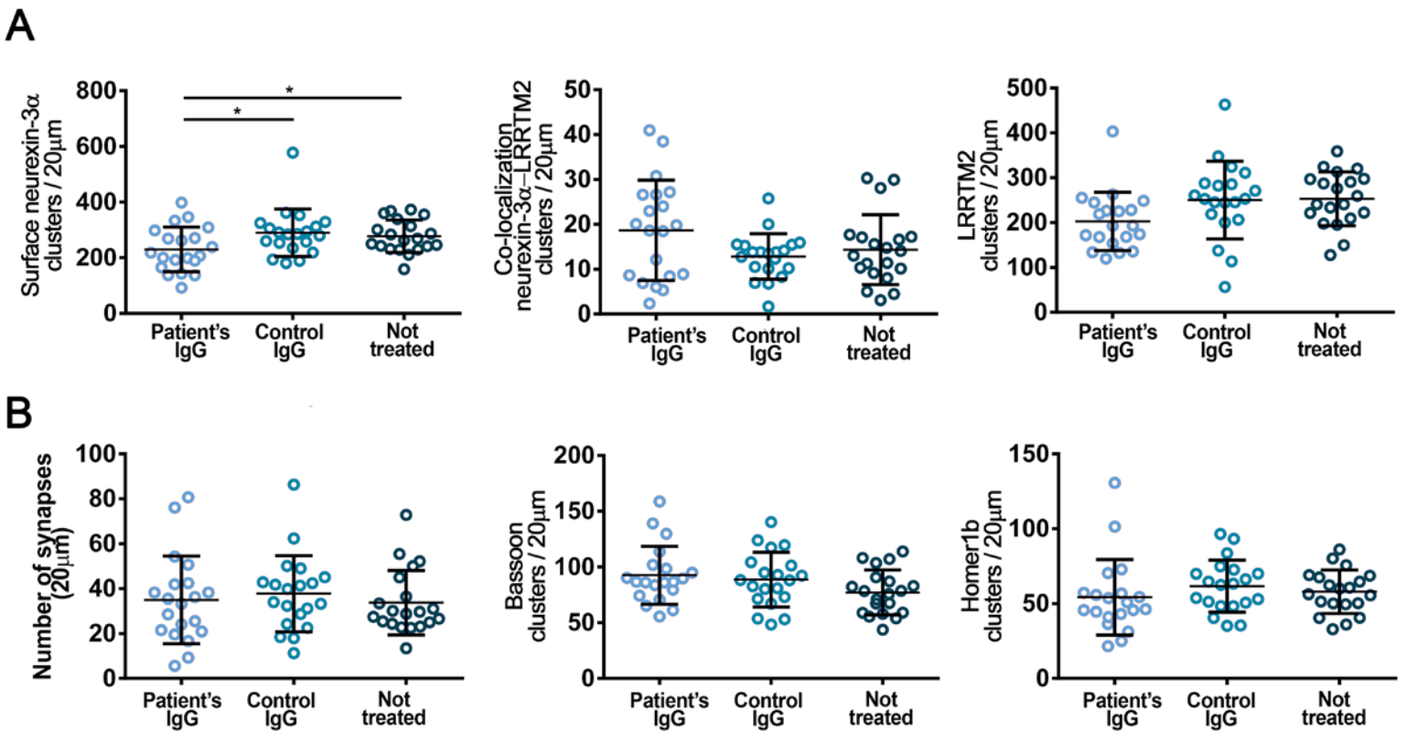
The reactivity of patients' antibodies with rat hippocampus (A) is abrogated after immunoabsorption with HEK293 expressing neurexin-3α (B) but not after immunoabsorption with non-transfected HEK293 cells (C). Scale bar = 500µm

Figure e-3: Patients' IgG antibodies do not affect the number and complexity of dendrites, or the density of dendritic spines.



(A) Number of dendrites classified by dendrite order (1st, 2nd, 3rd) plotted as percentage of the total number of dendrites. (B) Dendrite length (µm) classified by dendrite order. (C) Number of spines for 20µm of dendrite (only 2nd and 3rd order dendrites plotted). For all panels the light blue column represents neurons treated with patient's IgG, the middle blue column neurons treated with control IgG, and the dark blue column neurons not treated (not treated). All graphs represent mean ± SD of 3 independent experiments.

Figure e-4: Patients' IgG antibodies cause a reduction of neurexin-3α in mature neurons but do not affect the number of synapses.



(A) Mature neurons (*div* 18) treated with patients' IgG for 48 hours, but not control IgG or untreated neurons, showed a significant reduction of the density of clusters of cell-surface neurexin-3α (left), without change in the co-localization of neurexin-3α with its ligand LRRTM2 (middle) or the density of LRRTM (right). All graphs represent mean ± SD (20 dendrites per condition); *$p<0.05$.

(B) Mature neurons similarly treated as above show no significant reduction of the number of synapses (defined by the co-localization of the presynaptic marker bassoon with the postsynaptic marker homer1b; left), or the levels of bassoon (middle) or the levels of homer1b (right). All graphs represent mean ± SD (20 dendrites per condition).