SUPPLEMENTAL INFORMATION

Preparation of patients and control IgG

IgG was purified from patients with DPPX antibodies and healthy blood donors (controls) using protein A/G (Pierce™ Protein A/G agarose, Thermo Fisher Scientific, US) and filtered using copolymer styrene/butadiene filter (Millipore, Billerica, MA) following the manufacturer instructions. Before assessing the effects of patients' IgG antibodies on cultured neurons, 1) each purified patient IgG was tested for antibody reactivity with frozen rat brain sections, cultured neurons, and HEK293 cells expressing DPPX, and 2) we confirmed that the IgG reactivity in cultured neurons was directed against DPPX using immunoabsorption studies as reported[1, 2] (data not shown).

Identification of IgG subclass of patients' DPPX antibodies with CBA

Identification of DPPX-IgG class was performed using indirect immunostaining of HEK293 cells expressing DPPX.[3] After fixation with 4% paraformaldehyde and permeabilization with 0.3% Triton™ X-100, cells were blocked with 1% bovine serum albumin for 2 hours, and co-incubated with patients' or control serum diluted 1:40 and a mouse monoclonal antibody against DPPX (1:500; Santa Cruz Biotechnology, St. Cruz, USA) at 4°C overnight. Then, cells were washed with phosphate buffered saline (PBS) and incubated with specific FITC-conjugated secondary antibodies against the four human IgG classes (1:500; IgG1-IgG4, The Binding Site, Birmingham, England), and Alexa Fluoro® 594 Goat anti-mouse IgG (1:1000, Molecular Probes) for 1 hour at room temperature (RT).[4] Results were photographed using a confocal microscope (LSM710, Carl-Zeiss, Germany).

Analysis of antibody effects and recovery using confocal microscopy

Fourteen days in vitro rat hippocampal neurons were prepared from E18 embryos as previously described.[5] Neurons were plated at a density of 150,000 in 35mm plates pre-coated with poly-L-lysine/collagen. Cultured neurons were then treated with individual or pooled patients or control IgG for 3 days at 37°C. After 3 days incubation

with patients' or control sera the effects of patients antibodies on DPPX or Kv4.2 were determined (see below), or neurons were washed with fresh media and allow to recover for 4 or 7 days in media without patients antibodies.

To determine the effects of patients antibodies without or with 4-7 days recovery, neurons were washed in Neurobasal plus B27 and incubated with a patient's anti-DPPX purified IgG (used here as a reagent, as in[6]) for 30 minutes at 37º C, washed with Neurobasal plus B27, incubated with Alexa Fluoro® 488 Goat anti-human IgG (1:1000, Molecular Probes) for 1 hour at 37º C, and then fixed in 4% paraformaldehyde for 5 minutes. Images were obtained with a laser-scanning confocal microscope (LSM710, Carl Zeiss, Germany) with EC-Plan NEOFLUAR CS ×100/1.3 NA oil objective, 1024 × 1024 lateral resolution, and standardized z-stacks (0.08 μm × 35 slices). Images were deconvolved with maximum likelihood estimation algorithms of Huygens Essential software (Scientific Volume Imaging, Hilversum, Netherlands). Cluster density was analyzed using a spot detection algorithm equipped in Imaris suite Ver. 7.6.4 (Bitplane, Zürich, Switzerland).

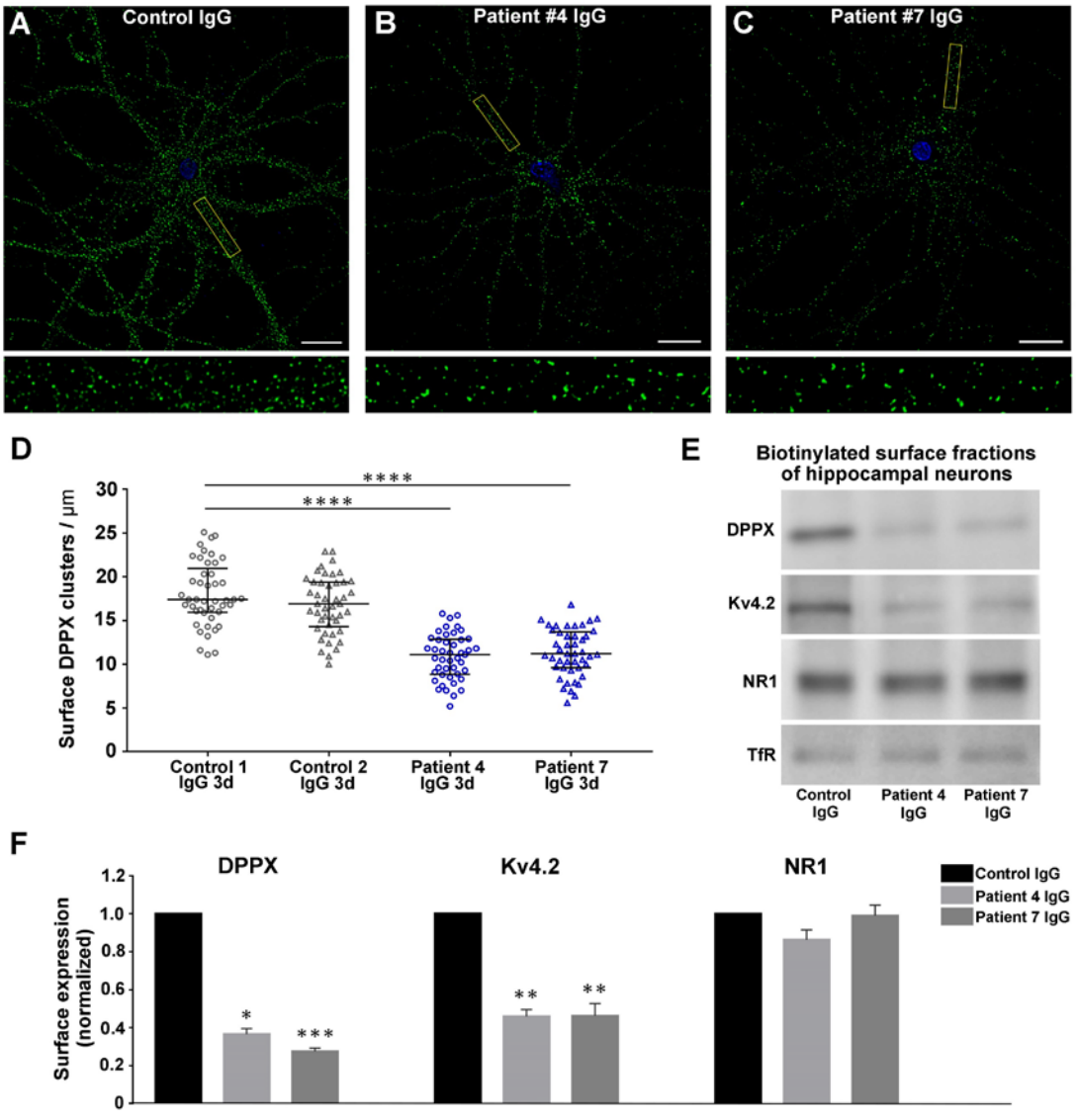
Analysis of antibody effects and recovery using immunoblot for biotinylated cell-surface proteins

To assess the effects of patients' antibodies on cell surface levels of Kv4.2, neurons plated at a density of 500,000 in 35mm plates were treated as above with patients' or control IgG for 3 days without or with the indicated period of recovery. Neurons were then washed twice in cold PBS (pH7.4), and incubated for 30 minutes at 4°C with 1mg/ml EZ Link Sulfo-NHS-SS-Biotin (Thermo Fisher Scientific) in cold PBS. Excess free biotin was quenched by incubating with cold PBS supplemented with 100mM Glycine for 20 minutes. Neurons were then rinsed in PBS and lysed with 150mM NaCl, 1mM EDTA, 100mM Tris HCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS containing protease cocktail inhibitor (diluted 1:50, Sigma, saint Louis, MO, USA) shaking for 1 hour at 4°C. Lysates were cleared of debris by centrifugation at 13,000 × g for 20 minutes, the supernatant was collected and the protein concentration measured using the bicinchoninic acid assay (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific). Equal amounts of biotinylated proteins from each IgG-treated neurons (900 μg) were then incubated with avidin-linked agarose beads (Pierce™ High Capacity Neutravidin Agarose, Thermo Fisher Scientific) at 4°C overnight. The beads were rinsed with a 3 column volume of PBS, and the surface fraction was eluted with 2× SDS loading buffer. The surface fraction was then analyzed by immunoblot. For each condition, equal amounts of proteins were loaded onto 8%

SDS-polyacrylamide gels and transferred to PVDF membrane. The membrane was blocked with 5% non-fat skimmed milk and incubated with the primary antibodies at 4°C overnight. The primary antibodies included, anti-GluN1 (1:1000, Catalog #G8913, Sigma-Aldrich), anti-DPPX (1:1000, Catalog #ab41811, Abcam), anti-Kv4.2 (1:300, Catalog #P0233, Sigma-Aldrich), anti-β-actin (1:20,000, Catalog #A1978, Sigma-Aldrich), and anti-transferrin receptor (1:2000, clone H68.4, Thermo Fisher Scientific). After incubation with primary antibodies, membranes were incubated with horseradish-peroxidase conjugated secondary antibodies (anti-rabbit IgG; 1;1000, or anti-mouse IgG; 1:10,000) for 1 hour at room temperature, and visualized with enhanced chemiluminescence (all Amersham GE Healthcare) on a LAS4000 (GE Healthcare). Protein concentrations were quantified by using scanning densitometry with Fiji Image J software. Surface expression of DPPX and Kv4.2 were normalized with that of the NMDA receptor subunit NR1, as a loading control.

Legend to Figure e-1

Figure e-1: Effects of patients' antibodies on cell surface DPPX and Kv4.2

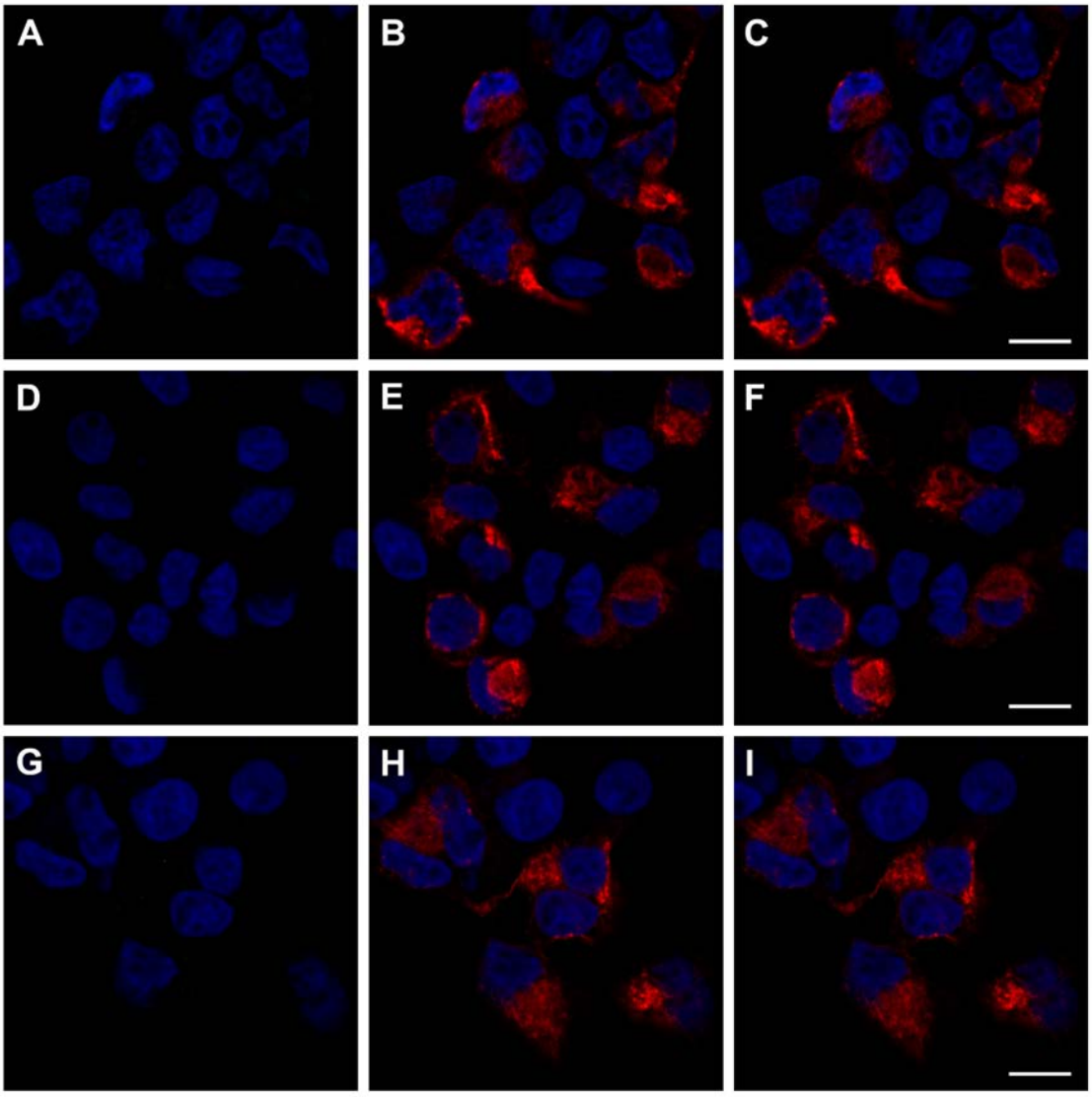


Panels A-C show 14 day-in-vitro cultured rat hippocampal neurons treated for 3 days with control IgG and patients 4 and 7 IgG; boxed dendrites are showed in the second row at higher magnification (×100/1.3 NA oil objective). Note that patients' DPPX antibodies result in a decrease of cell surface clusters; the smaller number but apparent

larger size of clusters shown in the enhanced dendrites likely represent antibody-mediated aggregates of clusters; Scale bars = 10 μm. Panel D, shows the graphic representation of the density of DPPX clusters (median with interquartile range) after treatment for 3 days with control or patients' IgG (3 independent experiments, 15 neurons per experiment in each condition). Panel E, shows immunoblot of biotinylated surface proteins of hippocampal neurons treated with control IgG, and patients 4 and 7 IgG for 3 days (as in A, B). The protein bands are demonstrated with commercial antibodies specific for DPPX and Kv4.2; NR1 and transferrin receptor are used as loading controls. Note that patient's antibodies cause a substantial decrease of levels of DPPX and Kv4.2. Panel F shows the quantitative densitometry analysis of E; the data was normalized to the values of control IgG and is represented as median with SEM of 3 independent experiments (for each experiment, immunoblots were repeated, n=6). All statistical analysis: Kruskal-Wallis test followed by Dunn's test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Legend to Figure e-2

Figure e-2: Analysis of patients' antibodies using a cell-based assay expressing Kv4.2



HEK 293 cells expressing Kv4.2 immunostained with sera of representative cases (patient 4; A, and 7; D), a healthy individual (G), and a rabbit polyclonal antibody (1:2000, Alomone labs, #APC-023) against Kv4.2 (B, E, H). The merged reactivities are shown in the corresponding panels (C, F, I). Note that patients' antibodies do not recognize Kv4.2.

Nuclei counterstained with 4’, 6-diamino-2-phenylindole (DAPI) (A-I). Scale bars = 10 μm.

Supplemental References

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3. Boronat A, Gelfand JM, Gresa-Arribas N, et al. Encephalitis and antibodies to dipeptidyl-peptidase-like protein-6, a subunit of Kv4.2 potassium channels. Ann Neurol 2013;73:120-128.

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5. 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.

6. Planaguma J, Leypoldt F, Mannara F, et al. Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. Brain 2015;138:94-109.