Supplementary Material 1

Appendix

HEK 293 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Sigma D6429) supplemented with 10% Fetal Calf Serum (FCS; Biowest S181B) and 1% penicillin G/streptomycin/amphotericin (PSA; Life technologies 15240-062) in flasks kept in a humidified, 5% CO2 incubator. For screening purposes, those cells grown in flasks were transferred to 6-well plates with 13 mm poly-l-lysine coated glass coverslips, by gentle detachment with 1% trypsin in phosphate buffer saline (PBS) and re-seeding in DMEM/10% FCS/1% PSA at a concentration of 2.5 x 105 cells/mL, in a total volume of 2 mL/well. The following day, cells were transfected using 1.5 l polyethylenimine (PEI) with 3 g of the cDNA plasmids encoding the relevant human subunits. After 14-16 hours, the medium was replaced with fresh DMEM/10% FCS/1% PSA. In the particular case of the NMDAR CBA, to prevent cytotoxicity as a result of glutamate in the medium activating the NMDARs, the medium was supplemented with 500 M of ketamine. Immunocytochemistry (ICC) was performed on the fourth day. Up until then, all procedures were conducted in sterile conditions in a class II tissue culture hood. For ICC, coverslips were transferred to individual wells in a 24-well plate. Patient serum samples were added to DMEM supplemented with bovine serum albumin (BSA) (1% w/v) and 200 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma H3375) at a 1:20 or 1:100 dilution ratio, to make a total volume of 250 μl/sample. Positive (known positive patients) and healthy controls were added in each assay. Diluted sera were incubated with the live cells on coverslips at room temperature for 1 hour, after which the supernatant was aspirated and washed 3 times in DMEM/HEPES. Cells were fixed in 4% formaldehyde in PBS and incubated for 10 minutes at RT and then washed 3 times in DMEM or PBS. Afterwards, cultures were incubated for 45 minutes in the dark, at room temperature, with goat anti-human IgG, Alexa Fluor 568 (Invitrogen; A21090; 1:750) in DMEM+HEPES+BSA. The coverslips were washed 4 times in PBS and mounted using fluorescent mounting media (Dako, S3023), containing 4',6'-diamidino-2-phenylindole (DAPI; Sigma D9542; 1:1000). After drying, cells were viewed with a Leica DM 2500 immunofluorescent microscope. Binding was then scored on a scale of 0-4 according to the intensity of the immunofluorescence (0= no binding, 1= weak binding, 2= moderate binding, 3=strong binding, 4=very strong binding). Any sample scoring 1 or above was repeated and double scored by a second, independent observer. If there was disagreement between observers, the sample would be retested. Positive samples (score ≥1) were then titrated and end-point titration determined (last titration at which a score=1 was still observed). Case and control samples were screened blinded to clinical status.

To confirm positive samples and the IgG nature of the binding, the live CBAs were repeated replacing goat anti-human IgG, Alexa Fluor 568 (Invitrogen; A21090; 1:750) with a goat anti-human Fc IgG(specific) secondary (Thermo Scientific; 31125; 1:750) followed by a mouse anti-goat IgG, Alexa fluor 568 (1:750) for 45 minutes at room temperature as mentioned in the methods. See Figure 1 for examples.



**Score 2.5**

**Score 0**