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Supplementary Materials for

Human endogenous retroviral protein triggers deficit in glutamate synapse maturation and behaviors associated with psychosis

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Supplementary Materials

Supplementary Materials and Methods

Cell cultures and transfection. Animal procedures were conducted in accordance with the European Community guidelines (Directive 2010/63/EU) regulating animal research, and were approved by the local Bordeaux Ethics Committee (APAFIS#3420-2015112610591204). Mixed cultures of hippocampal neurons and glia cells were prepared from E18 Sprague-Dawley rats. In brief, cells were plated at a density of 300-350 x 100 cells per dish on poly-lysine coated coverslips and were maintained in Gibco neurobasal medium (Thermo Fisher Scientific, Massachusetts, USA) containing 3% horse serum for approximately 4 days *in vitro* (div) at which the medium were changed to a serum-free neurobasal medium. Banker type "glia free" hippocampal cultures were prepared in two steps. Briefly, first glia feeder cultures were prepared in poly-lysine coated dishes from hippocampus then, after two weeks, hippocampal neurons (from the same type of preparation as for the glia cells) were cultured on poly-lysine coated coverslips which were suspended above the glia layer. Cells were kept at 37°C in 5% CO_2 for 22 div at maximum. Human embryonic kidney cells (HEK) 293 were plated on glass coverslips, and Cos7 cells (fibroblast derived from monkey kidney tissue) directly on the plastic in 6 well plates, in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific) with 10% fetal calf serum and used one day later.

Cells were transfected using either Effectene (Qiagen, Hilden, Germany) according to the manufacturer's recommendations or by phosphate calcium transfection. The plasmids used can be found in the Table S3. Verification of the phCMV-MSRV Env (clone pV14, AF 331500) plasmid expression can be observed in figure S3 and that of shTLR-4 in figure S2B. Transfection density (Fig. 3) was estimated by counting transfected cells in relation to DAPI stained cells in 5 randomized fields covering approximately 10% of the coverslip.

Electrophysiology. Isoflurane anesthetized rats at postnatal day (P)14 were sacrificed by decapitation, brains were rapidly removed and dissected. 350 µm thick sagittal slices were cut with a vibratome (Leica, VT 1000S) in an ice-cold sucrose based artificial cerebral spinal fluid (aCSF) containing in mM: 2 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 220 Sucrose, 10 D-Glucose, 0.2 $CaCl_2$ and 6 $MgCl_2$. Slices were then incubated in standard aCSF solution (124 NaCl, 3 KCl, 26

$NaHCO_3$, 1.25 NaH_2PO_4, 10 D-Glucose, 2 $CaCl_2$ and 1 $MgCl_2$ 295-305 mOsm) for 45–60 min at 33°C, and subsequently maintained at room temperature throughout the duration of the experiment. All solutions were constantly bubbled with carbogene (95% O_2, 5% CO_2) to maintain a neutral pH.

Electrophysiological recording. For electrophysiological recordings, individual slices were transferred to a heated submerged recording chamber (30.5 ± 0.5 °C) perfused with aCSF at 3 mL/min. Electrodes, of 4.5–5.5 MΩ resistance, were prepared from borosilicate pipettes (GC150T-10, Harvard Apparatus, San Diego, CA) with a vertical micropipette puller (PC-10, Narishige, London, UK). Intracellular solution contained in mM: 120 $CsMeSO_4$, 4 NaCl, 2 $MgCl_2$, 10 Hepes, 0.2 EGTA, 4 Na_2ATP, 0.3 Na_3GTP and 5 Phosphocreatine. PH was adjusted to 7.3-7.4 with CsOH. Whole-cell patch-clamp recordings of hippocampal CA1 pyramidal cells were performed using infrared differential interference contrast microscopy (Nikon, Tokyo, Japan) with a 60x water-immersion objective. All recordings were performed using an EPC10 USB amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany) sampled at 10 kHz. No correction of junction potential was applied. CA1 pyramidal neurons were recognized by their localization within the pyramidal layer and their firing properties. NMDA responses were recorded at a membrane potential of +40mV in the presence of 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-quinoxaline-7-sulfonamide (NBQX 10 µM, Tocris, Bristol, UK) and bicuculline (20µM, Tocris). Drugs were applied trough the perfusion system. Glutamatergic responses were evoked in stratum radiatum (SR) through monophasic electrical stimulation of the SR with a tungsten bipolar electrode (TST33A05KT Microelectrode Tungsten, World Precision Instruments, Sarasota, USA) controlled by an ISO-Flex stimulator (AMPI, Jerusalem, Israel). Single stimuli were delivered at 0.05 Hz and the responses were observed in pyramidal neurons as evoked EPSCs in voltage-clamp. Under these conditions and after a stable eEPSC recording had been maintained for 10 min, recombinant Env (1µg/ml) was added to the preparation, and responses recorded for 10 minutes prior to wash. Vehicle or boiled protein solution was used as control. The amplitudes and kinetics of eEPSCs were calculated from the baseline current preceding each individual stimulation artefact using Clampfit 10.1 (Molecular Devices, Sunnyvale, USA). For each cell, the median of 15 events amplitude peak before and 5 minutes after drug application was considered.

Immunocytochemistry. In general, live surface staining (10min at 4°C) was followed by 15 min fixation in 4% paraformaldehyde (PFA), quenching in 50mM NH_4Cl for 10min, blocking for 1h and incubation with secondary Ab's coupled to Alexa fluorophores for 1h in 1% bovine serum

albumin (BSA) (Sigma-Aldrich, Missouri, USA) at room temperature (R.T.) For staining of fixed cells and tissues, incubation overnight with primary antibodies (see Table S3) at 4°C was done after an initial block and permeabilization step in 1% BSA (Sigma-Aldrich) and 0.1% Triton X-100 (Sigma-Aldrich) in phosphate-buffered saline (PBS) for 1h. Surface or intracellular staining of Env in HEK-cells transfected with cytosolic enhanced green fluorescent protein (EGFP) and Env was obtained after 48h with an anti-Env Ab (GN_ ENV_01, GeNeuro) (Fig. S3). For the microglia activation study, cells were fixed and stained for Iba1 24h after LPS (1µg/ml, serotype O26:B6, Sigma-Aldrich), Env (1µg/ml, PX'Therapeutics) or saline (Control) application. Before human serum incubation, neurons (10 div) were transfected with either the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-A1 containing; the N-Methyl-D-Aspartate receptors (NMDA)-N1 containing; the gamma-Aminobutyric acid (GABA)-γ^2 containing subunits fused to the Super Ecliptic pHluorin (SEP) or the dopamine (D)-1 containing subunit fused to Cyan Fluorescent Protein (CFP). Serum samples from Env negative and Env positive subjects (5 out of 6 and patient 1-4 and 6,7 respectively (see Table S1), due to low amount of available biological material) were slowly thawed and, following incubation with serum samples at 20% for 15min at 37°C, live immunostaining of surface receptors were conducted with an anti-GFP antibody (see Table S3) for 10 min at 4°C (heat inactivation of complement factors in serum samples was considered but not applied due to the known heat sensitivity of human Env-protein). Cells were mounted in Mowiol (Calbiochem, Merck) or Vectashield® + DAPI (Vector Laboratories, Burlingame, CA).

Images were randomly selected for analysis and either collected on a video confocal spinning-disk system (Leica DMI6000B, Wetzlar, Germany) with a CoolSNAP HQ2 camera (Photometrics, Tucson, USA) or, on a Nanozoomer (Hamamatsu, Japan). For surface cluster analysis, dendritic branches were chosen manually (ROIs) in a blinded manner and cluster areas and numbers were obtained using a manual threshold approach based on integrated fluorescence levels in ImageJ (NIH). Dendritic density of glutamatergic synapses was calculated as number of endogenous PSD-95 positive or exogenous Homer 1c clusters/dendritic length. Microglia perimeter (µm) and area (μm^2) was obtained from all microglia present on entire coverslips and a transformation index (TI) was calculated: (perimeter of cell) 2 / 4p*i* (cell area).

ELISA multiplex. Cytokine levels were examined using a Milliplex Map Kit (RECYTMAG-65K, Millipore, Burlington, USA). Culture medium, from 3 different cultures, collected 5 min after vehicle (Control) or Env (10µg/ml) application were processed according to the

manufacturer's recommendations and mean fluorescent intensities were obtained using a Luminex xPONENT software on a Bioplex® MAGPIX reader (BioRad, Hercules, USA). Data was normalized to control within each experiment.

Tissue Preparation. Brains were removed at P7 or 2-14 days after PPI and either the whole brain was rapidly frozen in isopentane (Sigma-Aldrich) and placed in liquid nitrogen or, it was transferred to ice cold artificial cerebral fluid for dissection of the hippocampal areas and then frozen in liquid nitrogen. The frozen hippocampal tissue was later processed for biochemistry as described below. For immunohistochemistry, 20 μm thick coronal tissue sections from the whole brains were cut on a microtome-cryostat (Leica CM3050S), thaw-mounted onto superfrost ultra plus (Thermo Scientific) slides, and stored at –20°C until further processing. A few animals at age P7: Control (n = 5) and Env (n = 5), and at age P59-70: Control (n = 5) Env (n = 5) were anesthetized with pentobarbital (50 mg/kg) and transcardially perfused with 4% PFA. Brains were removed and postfixed overnight at 4°C and 50-μm-thick slices were prepared with a VT1200S Leica vibratome. Slices were washed three times with PBS and left in 0.03% acid-PBS at 4°C for later use.

Immunohistochemistry/TUNEL Staining. Perfused tissue was blocked and permeabilized in 4% BSA (Sigma-Aldrich) and 0.2% Triton X-100 (Sigma-Aldrich) for 2h at R.T. After rinsing, samples were incubated with primary Ab's (see Table S3) in 2% BSA (Sigma-Aldrich) and 0.2% Triton X-100 (Sigma-Aldrich) overnight at 4°C. Secondary Ab's coupled to Alexa fluorophores were incubated for 2h at R.T. in the same solution as the primary Ab`s. Sections were mounted using Mowiol (Calbiochem) or Vectashield® + DAPI (Vector Laboratories, Burlingame, CA). DNA fragmentation was examined histologically using the in situ Apoptosis Detection System Fluorescein (TUNEL, Promega, Madison, USA). Briefly, frozen tissue sections from P7 electroporated animals were stained according to the manufacturer's recommendations and mounted with Mowiol (Calbiochem). All images were collected on a video confocal spinning-disk system (Leica DMI6000B, 63X) with a CoolSNAP HQ2 camera (Photometrics) or on a Nanozoomer (Hamamatsu). The measure of microglia density was blindly performed and microglia density was measured in the stratum radiatum/lacunoso moleculare (SR) of CA1 hippocampal region. Microglia density was determined by dividing the manually counted cells by the analyzed area expressed in mm^2. Only Iba1 cells with identifiable nuclear staining were considered and put in relation to all DAPI positive stained nucleuses (% Iba1 positive cells).

Western blot analyses. Dissected hippocampus from control and Env electroporated rats (P7 and ~P65-70) were processed by subcellular fractionation, including multiple centrifugations and a final ultra-centrifugation step (Fig. 5A), to finally solely collect the synaptic enriched fraction (synaptosomes: synapses, synaptic plasma membranes and synaptic vesicles). Protease and phosphates inhibitors (Thermo Ficher Scientific) were added to the isotonic sucrose for homogenization and fractionation. The protein concentration of each sample was determined with Pierce BCA Protein Assay Kit (Thermo Ficher Scientific) and synaptosomes from 9 animals per condition were examined on two separate experiments. For GFP detection whole hippocampus homogenates were used.

We used a Wes™ protein simple apparatus (Protein simple, bio-techne, San Jose, USA) and WES-total protein pack, plus WES-standard pack (12-230 kDa) including anti rabbit secondary or anti mouse antibody. GluN2A, GluN2B or GluA2 detection in relation to PSD-95 was measured (Fig. S7A-D). 0.1µg of each sample was loaded and primary antibodies, rabbit anti-GluN2(A and B) (Agrobio, specific custom-made, Fig. S7A,B) and anti-PSD-95 (Cell Signaling, Danvers, USA), see Table S3. Only values within the range of two standard deviations were included for further analysis. GFP was detected by conventional western blotting. Briefly, 20µg of total protein was loaded in each lane separated with 4-20% precast SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, USA). The membrane was blocked in 5% non-fat Milk Tris-buffered saline (TBS)/0.1% Tween 20 (TBST) at R.T. for 1 h. Primary antibody mouse anti-GFP (Roche, Basel, Switzerland) were diluted in 0.5 % Milk in TBST for protein immunoblot analysis and incubated O.N. at 4°C under agitation. Incubation with horse radish peroxidase (HRP)-conjugated anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, USA) was performed for 2 h at R.T. Specific protein bands were revealed with Clarity™ Western ECL Substrate (Bio-Rad) and the membranes were scanned using a Versadoc apparatus (Bio-Rad).

Immunoprecipitation. Dynabeads Protein A (Invitrogen, Carlsbad, California, USA) was used following the manufacturer's recommendations. In brief, were incubated for 30min at 4ºC under rotation with a rabbit anti-IL-1R antibody (Santa Cruz, Dallas, Texas, USA). Synaptosomes (see above, 50 µg) from P7 and P65 animals was then added and rotated overnight at 4ºC. Supernatant was removed and saved, and immunoprecipitates were washed three times in lysis buffer. SDS–PAGE buffer was added to the washed immunoprecipitates, which then were resolved on 7%

precast SDS-polyacrylamide gels (Bio-Rad). Efficiency of the immunoprecipitation was determined by examining the supernatant and wash fractions obtained from the procedure on images obtained from a Versadoc apparatus (Bio-Rad) (see western blots section). Band density values for coimmunoprecipitated GluN2A and GluN2B were normalized to immunoprecipitated IL-1R.

Study Participants. Written informed consent was obtained from all participating subjects and the institutional ethical committee approved the research protocol. Patients with bipolar disorder or schizophrenia meeting the DSM-IV criteria (APA, 1994) were included for an acute episode in the department of psychiatry of Henri Mondor hospital in Creteil, France, under the framework of an ongoing research program (I-GIVE, 13-SAMA-0004-01). Patients were interviewed with the French version of the "Diagnostic Interview for Genetic Studies" (DIGS) for the assessment of lifetime psychiatric disorders as well as for demographic characteristics. For this study, 7 patients (5 with bipolar disorder and 2 with schizophrenia) with high plasma levels of envelope protein (Env), were selected. Manic and depressive symptoms were assessed using, respectively, the Young Mania Rating Scale (YMRS) and the Montgomery-Åsberg Depression Rating Scale (MADRS) for bipolar disorder. Schizophrenic symptomatology was assessed using the Positive And Negative Syndrome Scale (PANSS), see individual patient information in Table S1. Env negative subjects (n = 6), belonging to the healthy control sample, were enrolled through a clinical investigation center (Centre for Biological Resources, Mondor Hospital, Créteil, France). Only controls without any personal or family history of psychotic disorders, affective disorders, addictive or suicidal behavior, or autoimmune diseases were included. Blood samples were collected from patient and control groups within one week of the clinical assessment. Patients and controls were submitted to serological screening and were negative for HIV-1/-2, Hepatitis A, B and C and had no known recent inflammatory, autoimmune disorder, infectious event or a neurological disease at inclusion. Individual participant blood sample data can be found in Table S1 which revealed a significant increase of IL-1β and B-cell type M2 (BM2) in Env positive patients compared to controls.

HERV-W Env quantification by sandwich ELISA. Microtiter plates were coated with two MSRV-Env monoclonal antibodies from GeNeuro (2.5µg/ml GN_ENV_04/well and 2.5µg/ml GN_ENV_16/well) or without antibody (uncoated well) overnight at 4°C. The plates were then washed in PBS-0.5% Tween 20 and blocked with 2% BSA solution for 1h at 37°C before sample

incubation for 1 hour at 37°C. The plates were then washed 4 times and the detection antibody, GN_ENV_01 (GeNeuro) conjugated to HRP was added for 1 hour at 37 °C. After 4 washes, the HRP substrate (3,3′,5,5′-Tetramethylbenzidine, Sigma-Aldrich) was added and plates were incubated for 30 min in the dark at R.T.. The colorimetric reaction was stopped with 1N sulfuric acid and the optical density read at 450 nm on a Plate Reader (BEP III, Siemens Healthineers, Erlangen, Germany). Protein results were expressed as HERV-W Env optical density (O.D.) at 450 nm. The unspecific adsorption of each sample, corresponding to the incubation of sample in uncoated well, was subtracted to O.D. of the respective coated well. The positive threshold was determined as the mean of O.D. of all negative samples plus 2 times its standard deviation (mean + 2SD). Quantification was calculated based on a standard curve using the *E. coli* recombinant monomer and therefore does not represent the real quantity of native antigen, it is thus an approximation of the concentration (Table S1).

Supplementary Figures and Tables

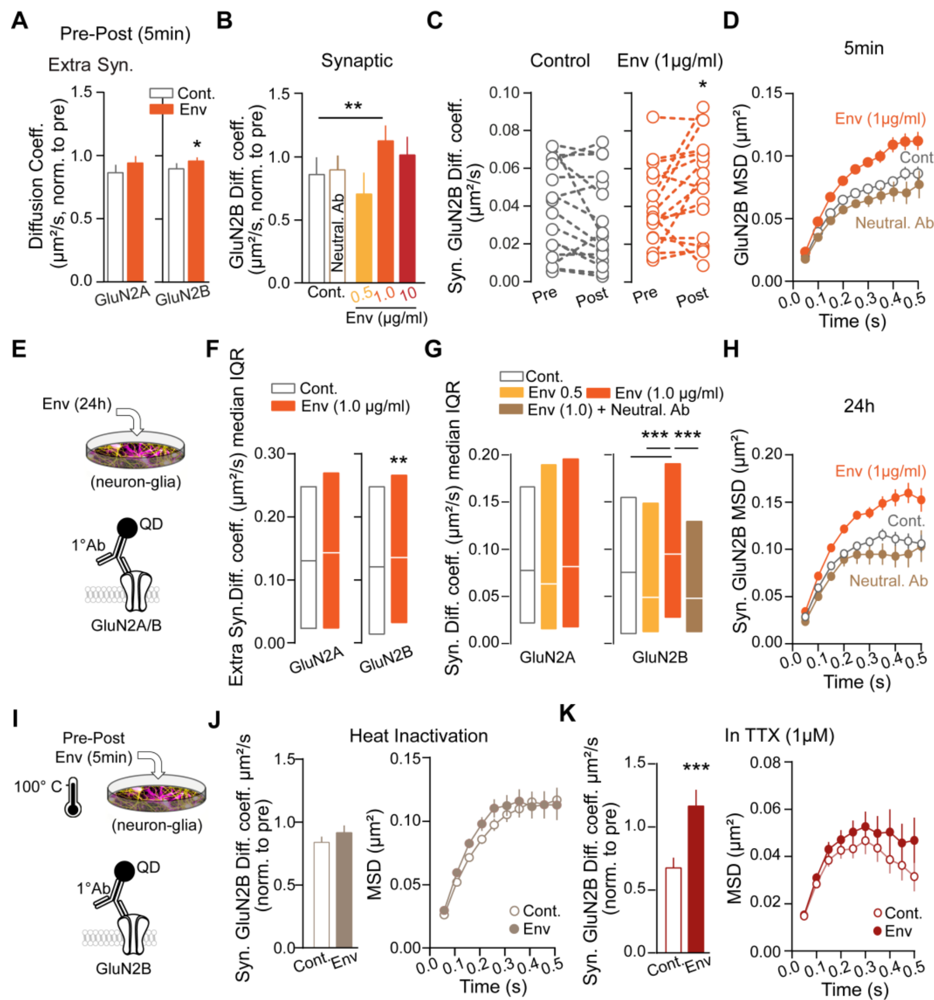

Fig. S1. The HERV-Env increase of synaptic GluN2B-NMDAR surface diffusion is maintained over time and independent from neuronal activity. (**A**) Extasynaptic GluN2A- and GluN2B-NMDAR surface diffusion 5 min after vehicle (Cont.) or Env (1µg/ml) exposure. GluN2A: Cont. (n = 300/3 trajectories/neurons), Env (n = 445/4) and GluN2B: Cont. (n = 819/10), Env (n = 727/9). **P* = 0.032, Mann-Whitney tests. (**B**) Synaptic GluN2B-NMDAR diffusion 5 min after Env application at different doses. Note that Env neutralizing Ab does not interfere with basal GluN2B-NMDAR diffusion. Cont. (n = 164/8 trajectories/neurons), Cont.+ Neutral. Ab (n = 261/5), Env: 0.5µg/ml (n = 90/7), 1µg/ml (n = 204/6), 10µg/ml (n = 140/7). ***P* = 0.002 from Kruskal-Wallis test followed by Dunn's multiple comparisons. (**C**) Median diffusion coefficient for synaptic GluN2B-NMDAR trajectories from individual neurons (circles). Paired data is shown for pre-exposure and 5min after Cont. (n = 16/12 neurons/cultures), or Env (1µg/ml, n = 17/13) addition. **P* = 0,038, paired t-tests. (**D**) Mean square displacements (MSDs)

for data in C and main Fig. 2E and 2F. (**E**) Experimental setup, prolonged Env exposure. (**F**) Extrasynaptic surface diffusion of GluN2A- and GluN2B-NMDAR after 24h Env exposure. GluN2A: Cont. (n = 2214/28), Env (n = 1670/31), GluN2B: Cont. (n = 3882/65), Env (n = 3872/51). $^{**}P = 0{,}003$, Mann-Whitney test. (**G**) Specific increase of synaptic GluN2B containing NMDAR-receptors surface mobility after prolonged Env exposure. GluN2A: Cont. (n = 382/16 trajectories/neurons), Env: 0.5µg/ml (n = 147/6), 1.0µg/ml (n = 277/16). GluN2B: Cont. (n = 612/22), Env: 0.5µg/ml (n = 485/19), Env: 1.0µg/ml (n = 600/18), Env + Neutralizing Ab (n = 204/14). $^{***}P < 0{,}0001$, Kruskal-Wallis test followed by Dunn's multiple comparisons. Data are median interquartile range (IQR). (**H**) Mean square displacements (MSDs) for GluN2B data in (G). (**I**) Experimental setup. (**J**) Stable GluN2B dynamics after heat inactivated Cont. (n = 563/7) or Env (n = 443/5). (*right*) MSDs for synaptic data. (**K**) Co-application of tetradotoxin (TTX, 1µM) and Env do not alter the Env effect, Cont. + TTX (n = 181/3) and Env + TTX (n = 182/4). $^{***}P = 0.0008$, Mann-Whitney test. Data shown are diffusion coefficients normalized to pre-condition for each individual neuron, mean ± SEM, if no other mentioned.

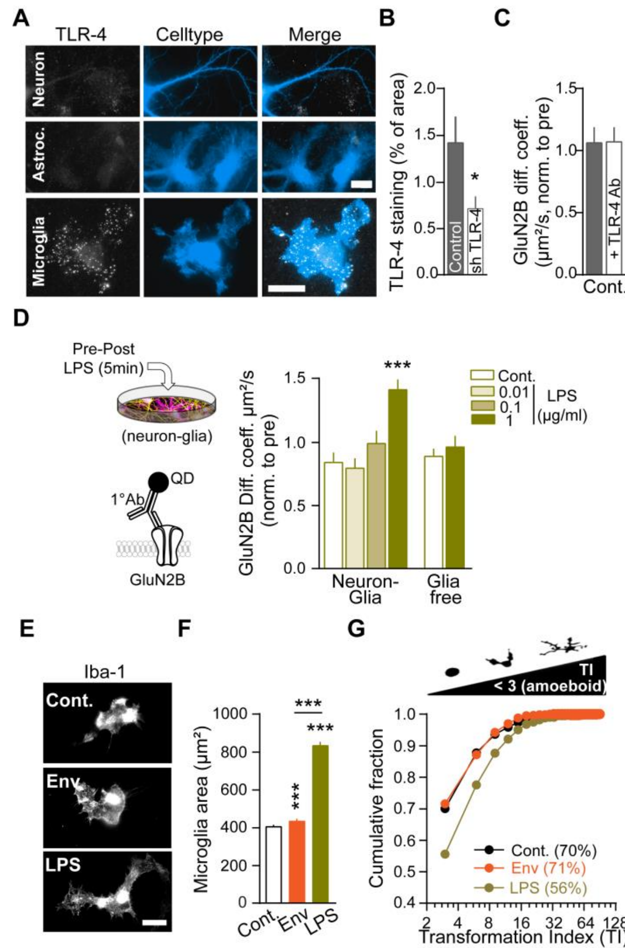

Fig. S2. LPS triggers increased synaptic GluN2B surface dynamics and distinct microglial activation compared to Env. Specificity of TLR-4 antibody; (**A**) Representative images with TLR-4 Ab staining (white) on GFP-transfected cells co-stained with cell type marker (blue; neurons (MAP-2), astrocytes (GFAP), microglia (Iba1). Scale bar = 10µm. (**B**) % of cell area occupied by TLR-4 staining. Control (GFP) transfected cells (n = 10 cells), shRNA (GFP + TLR-4 shRNA) transfected cells (n = 13 cells). (**C**) The neutralizing of TLR-4 with TLR-4 Ab does not interfere with GluN2B surface diffusion. Cont. (n = 168/4, trajectories/neurons), Cont.+TLR-4 Ab (n = 163/5). LPS stimulation; (**D**) Experimental setup. Increase in synaptic GluN2B-NMDAR diffusion in mixed, not glia free, hippocampal networks 5 min after lipopolysaccharide (LPS) stimulation. Neuron-glia: Cont. (Saline, n = 340/6, trajectories/neurons), LPS: 0.01µg/ml (n = 281/5), 0.1µg/ml (n = 329/5), 1µg/ml (n = 446/6). Glia free: Cont. (n = 547/4), LPS (1µg/ml, n = 312/4). Data are normalized to pre-exposure for each individual neuron, mean ± SEM. ****P* = 0,0004, Kruskal-Wallis test followed by Dunn's multiple comparisons. (**E**) Representative images of Iba1 positive microglia cells 24h after Env (1µg/ml) or LPS (1µg/ml) exposure. Scale bar = 10µm. (**F**) Quantification of microglial cell areas (µm²) 24h after: Cont. (Saline, n = 1763 cells),

Env (n = 1420) and LPS (n = 1279). ***P < 0,0001 from Kruskal-Wallis test followed by Dunn's multiple comparisons. (**G**) *(top)* Graphic illustration of microglia morphology in relation to transformation index (TI). *(below)* Cumulative fraction of TI, with values < 3 defined as amoeboid cells. Note the change in percentage of amoeboid cells after LPS stimuli (56%) compared to Cont. (70%) and Env (71%).

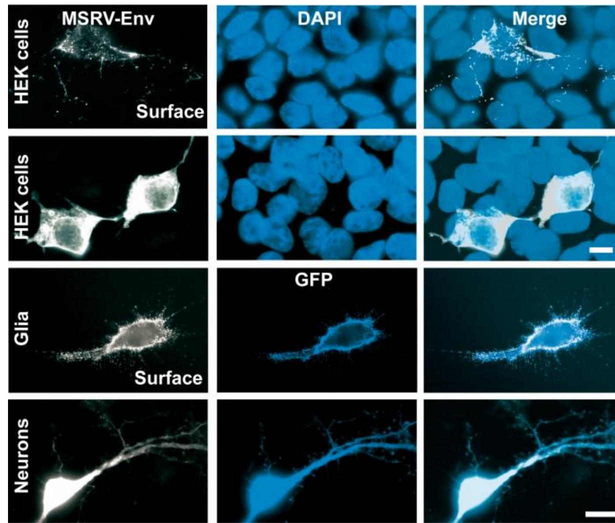

Fig. S3. Env expression in different Env-gene transfected cells types. Surface Env expression after live immunostaining or intracellular expression following fixation and permeabilization in HEK 293, glia cells and neurons. Scale bars = 10µm.

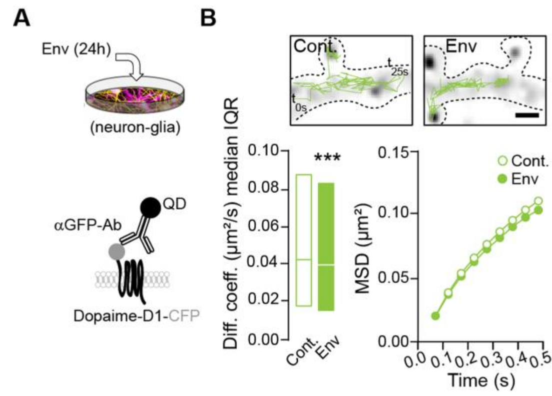

Fig. S4. The HERV-Env decrease dopamine-D1 surface diffusion. (**A**) Experimental setup. (**B**) Dopamine-D1 surface diffusion after 24h Cont. or Env exposure. Cont. (n = 14211/24 trajectories/neurons), Env (n = 9129/24). ****P* = < 0.0001, Mann-Whitney test. Scale bar = 1µm.

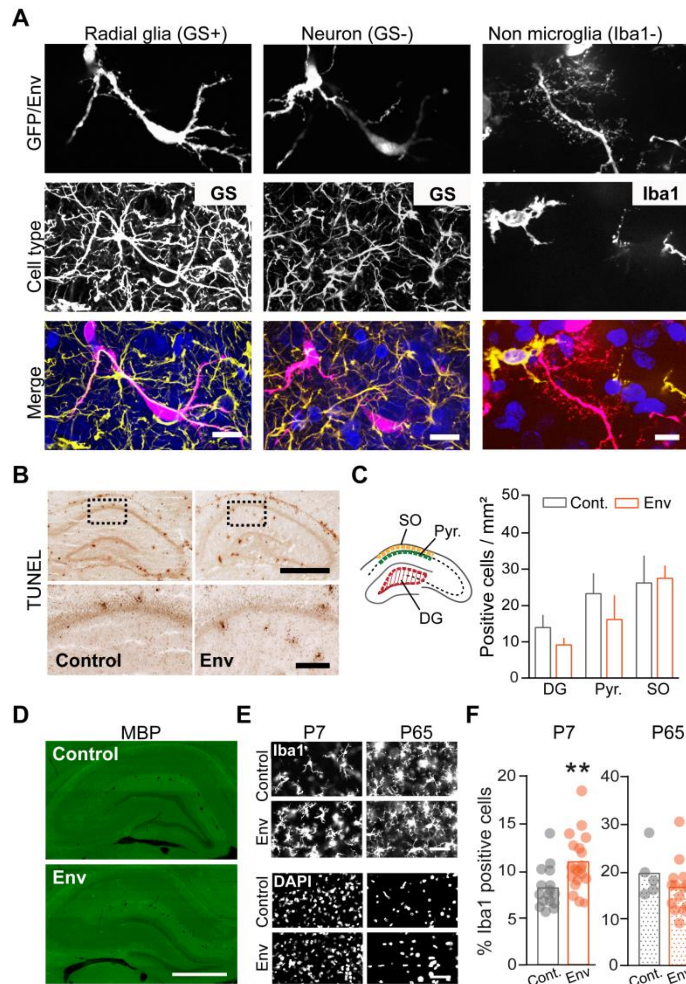

Fig. S5. Electroporated hippocampal cells are characterized as astrocyte/radial glial (GS+) and neuronal/non-glial (GS-, Iba1-) cells and devoid of cell survival decline. Coronal sections of hippocampal CA1 areas from electroporated animals at P7 (**A**) co-stained for GFP and glutamine synthetase (GS) or ionized calcium-binding adapter molecule 1 (Iba1). Scale bars = 10µm. (**B**) Representative images of apoptotic cell detection (TUNEL). Scale bars = 1mm and 200µm. (**C**) Quantification of TUNEL positive cells/mm² in different hippocampal regions. Values represent mean ± SEM, n = 3 animals/group. (**D**) Representative images of myelin basic protein (MBP) stained hippocampal sections. Scale bar = 1mm. (**E**) Representative images of Iba1 stained microglia with identified nuclear (DAPI) staining in CA1 stratum radiatum. Scale bars 50µm. (**F**) The number of Iba1 positive cells is increased in Env-animals at P7 (Cont. n = 16 hippocampi; Env, n = 20) but not at ~P65 (Cont. n = 5; Env, n = 14), **P = 0.005, Student's t-test.

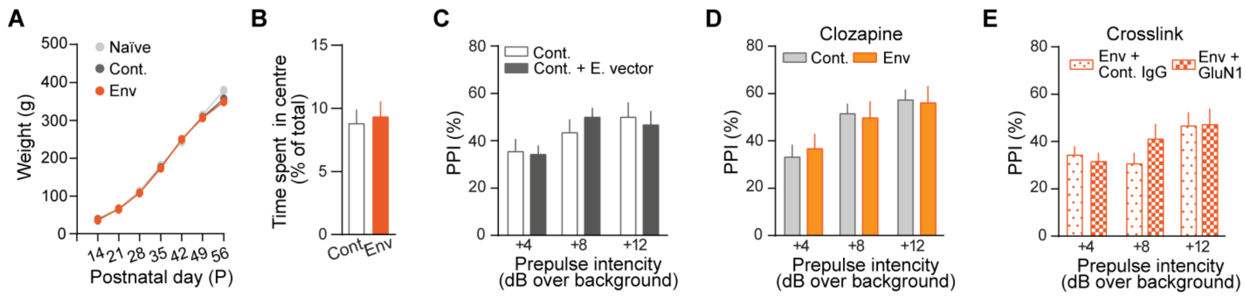

Fig. S6. Hippocampal HERV-Env effects *in vivo*: gene expression *per se* has no effect on weight, anxiety nor PPI response. (**A**) Normal weight gain in electroporated animals compared to naïve animals (n = 17-31 animals/group). (**B**) The Env-rats show no alteration in anxiety-like behaviour measured as time spent in centre of the open field (n = 16). (**C**) No influence of DNA load on pre-pulse inhibition (PPI) response (Cont. n = 11, Cont. + Empty vector n = 14). (**D**) Clozapine (12mg/kg) improves the PPI response in Env animals (n = 12). (**E**) PPI response in Control-animals after crosslink protocol with either Cont. IgG or GluN1-Ab (Cont. IgG n = 12, GluN1 n = 11). Values are mean ± SEM.

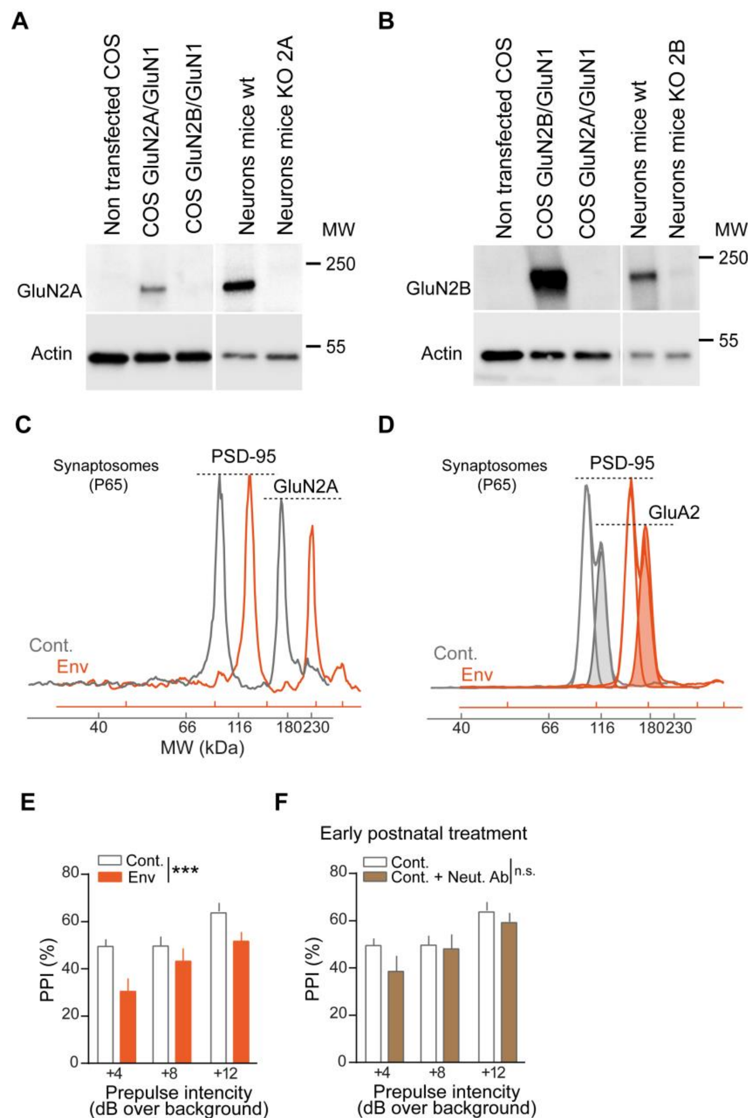

Fig. S7. Characterization of specific custom-made antibodies, WES protein expression and no effect on behavioural response in the PPI-test by early postnatal Ab-injections in control animals. Characterization of custom-made (**A**) GluN2A and (**B**) GluN2B polyclonal antibodies specificity by western-blot using non transfected COS-cells, COS-cells electroporated either with GluN2A/GluN1 or GluN2B/GluN1 and cultured neurons from WT mice and GluN2A knockout (KO) or GluN2B KO mice. The total amounts of protein loaded were validated with α-actin detection. Specific labelling and no cross-reactivity with the other subunit are observed. (**C,D**) Representative electropherograms from synaptosomes probed on a WES™-apparatus for NMDA(GluN2A)/PSD-95 and AMPA(GluA2)/PSD-95. (**E**) Env impairs PPI startle response (control experiments for treatment protocol). Group factor: $F(1,66) = 12.31$, $^{***}P = 0.0008$, two-way ANOVA, Bonferroni's multiple comparisons test, ($n = 12$). (**F**) PPI response in control

animals is not affected by early postnatal intra peritoneal treatment with Env neutralizing-Ab. Group factor: $F(1,69) = 1.80$, two-way ANOVA (n = 12-13).

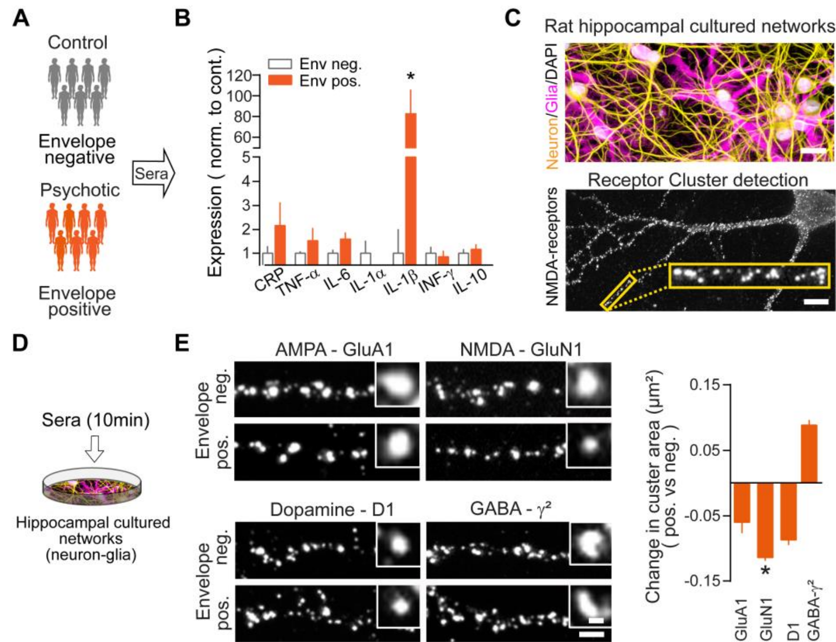

Fig. S8. HERV Env from sera of patients with psychosis alter NMDAR surface organization. (**A** and **B**) Increase of interleukin-1β in envelope-positive (pos., n = 7) human serum samples compared to (neg., n = 6). *P = 0.016, Student's t-test. (**C**) Representative images of (*top*) cultured hippocampal networks (yellow) neurons (MAP-2 positive) and (magenta) glia (GFAP and Iba1 positive) and, (*bottom*) a NMDAR-GluN1-transfected neuron. Scale bars, top/bottom = 30/10µm. (**D**) Experimental setup. (**E**) Env-positive serum-samples specifically decrease GluN1 cluster areas (insets). GluA1 (n = 57/43 neurons neg./pos.), GluN1 (n = 56/61), Dopamine1 (n = 54/61) and $GABA_{\gamma 2}$ (n = 59/68). Scale bars = 1µm and inset 0.3µm. *P = 0.048, Student's t-test, mean ± SEM.

Table S1. Participant information and data

Individual patient information															
	Gender	Age	Status	Polarity	Visit	MADRS_Tot	YMRS_Tot	Total_PANSS	PANSS_Pos	PANSS_Neg	PANSS_Gen	CGI	GAF_SYMP	GAF_HANDICAP	FAST_Total
Patient 1	F	30	acute SZC	N/A	1	6	19	82	24	26	32	5	28	32	59
					3	2	1	47	9	14	24	3	70	65	20
Patient 2	M	21	acute Bipolar	Manic	1	10	31	72	27	10	35	5	40	40	36
					3	5	4	41	8	9	24	2	71	71	10
Patient 3	M	42	acute Bipolar	Manic	1	9	3	108	27	27	54		33	33	59
					3	0	0	72	16	23	33	4	55	55	56
Patient 4	M	32	acute SZC	N/A	1	9	23	84	23	19	42		25	25	
					3	0	0					2			17
Patient 5	M	31	acute Bipolar	Manic	1	14	27								
					3										
Patient 6	F	66	acute Bipolar	Manic	1										
					3										
Patient 7	F	34	acute Bipolar	Mixed (Manic)	1	17	20	57	17	9	31	5	30	30	38
					3										

Individual participant data															
	Gender	Age	Visit	BAFF	BM2 (*P)	CRP	TNF-α	IL-6	IL-1α	IL-1β (*P)	IFN-γ	IL-10	IL-12p40	IL-17	Env (µg/ml)*
Patient 1	F	30	1	728	1.17	1025044.14	0.73	0.60	0.00	0.31	8.06	0.25	27.19	0.75	0.0719
			3		1.44										0.0428
Patient 2	M	21	1	823	1.43	5799527.19	1.97	0.50	0.02	0.05	2.06	0.34	67.23	0.66	0.1154
			3		1.61										0.1639
Patient 3	M	42	1	815	1.23	36763586.65	6.76	0.81	0.00	0.22	4.34	0.35	36.40	0.70	0.0973
			3		1.52										> 0.188
Patient 4	M	32	1	766	1.78	6102431.02	0.87	0.14	0.00	0.00	0.43	0.04	59.42	1.20	0.0626
			3		1.76										0.0517
Patient 5	M	31	1	933	2.39	7010470.89	2.62	0.77	0.00	0.09	2.93	0.35	72.98	1.75	0.0533
			3		1.76										0.0424
Patient 6	F	66	1	725	1.93	374413.61	1.24	0.21	0.00	0.19	1.86	0.10	20.18	0.85	0.176
			3		2.64										> 0.188
Patient 7	F	34	1	511	1.63	32685801.09	2.59	0.88	0.00	0.10	7.18	0.30	99.80	1.24	0.0594
			3		1.54										0.0495
	Gender	Age	Visit	BAFF	BM2	CRP	TNF-α	IL-6	IL-1α	IL-1β	IFN-γ	IL-10	IL-12p40	IL-17	Env (µg/ml)
Control 1	M	34	1	597	0.89	1643824.50	1.94	0.32	0.00	0.00	4.76	0.15	50.02	0.45	below LLOQ
Control 2	F	36	1	789	1.25	12996743.68	2.19	0.23	0.00	0.00	6.19	0.3	88	1.27	below LLOQ
Control 3	F	24	1	717	0.82	6201588.00	1.76	0.47	0.08	0.00	10.47	0.28	53.89	1.19	below LLOQ
Control 4§	F	47	1			9281560.84	1.45	0.62	0.16	0.01	3.04	0.30	99.88	1.06	below LLOQ
Control 5	F	36	1	911	1.22	3387385.23	1.33	0.34	0.01	0.00	2.89	0.3	86.19	2.31	below LLOQ
Control 6	M	33	1	440	1.46	2624758.89	1.50	0.35	0.04	0.00	1.74	0.07	29.51	0.29	below LLOQ

*The quantification was calculated based on a standard curve using the *E. coli* recombinant monomer and therefore does not represent the real quantity of native antigen. It is thus an approximation of the concentration. §This control was encountered with suspicion of inflammatory disease (Crohn's disease) after conduction of experiments in this work. Significant difference between mean of Control individuals and Env-positive patients is shown by *P = 0.0144 (BM2) and *P = 0.016 (IL-1β), Student's t-test. BAFF, B-cell activating factor; BM2, B-cell type M2; CGI, Clinical Global Impressions; FAST, Global Functioning Assessment Short

Test; GAF, Global Assessment of Functioning; IFN, Interferon; IL, Interleukin; LLOQ, Lower Limit Of Quantification; MADRS, Montgomery-Åsberg Depression Rating Scale; PANSS, Positive And Negative Syndrome Scale; SZC, schizophrenic; TNF, Tumor necrosis factor; YMRS, Young Mania Rating Scale.

Table S2. Statistical details

Main Figures	Parameters	Sample size	Nr. Rep. exp.	Sample collection	Sample collection-Conditions	Values	Statistical (n) value	Statistical test(s)	P-value (alpha = 0.05)	Assumptions and corrections
Figure 1B	Ratio Ca^{2+} transients frequency (Hz, Post/Pre)	n = 3-5 (cultures)		Blank, n = 5 Control, n = 7 Env (0.5µg/ml), n = 6 Env (1.0µg/ml), n = 9 Env (10µg/ml), n = 1 (neurons)	Blank, n = 47 Control, n = 92 Env (0.5µg/ml), n = 87 Env (1.0µg/ml), n =114 Env (10µg/ml), n = 18 (spines)	0.539 ± 0,057 0,489 ± 0,048 0.579 ± 0,049 0,604 ± 0,031 0,479 ± 0,061 (mean ± s.e.m.)	n = spines	D'Agostino & Pearson omnibus normality test followed by Kruskal-Wallis test	Kruskal-Wallis statistic = 8.931 P = 0.0629	Non-gaussian
Figure 1C	e EPSC amlpitude (pA)	n = 4-6 (animals)		Control, (n = 4) Env, (n = 6) (slices)	Control, (n = 4) Env, (n = 6) (neurons)	Dots represent mean amplitude eEPSC (pA)	n = paired neurons	Two tailed, paired t-test's	Cont. pre vs post, P = 0.36 Env pre vs post, P = 0.59	Gaussian
Figure 1E	Synaptic GluN2A and GluN2B instantaneous diffusion coefficient ($\mu m^2/s$) (normalized to baseline(pre)-condition for each neuron)	n = 3-13 (cultures)		*GluN2A:* Cont., n = 4, Env, n = 5 *GluN2B:* Cont., n = 16, Env, n = 17 (neurons)	*GluN2A:* *Synaptic:* Cont., n = 176 Env, n = 200 *GluN2B:* *Synaptic:* Cont., n = 670 Env, n = 792 (trajectories)	0.791 ± 0.083 0.895 ± 0.094 0.946 ± 0.065 1.193 ± 0.061 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney t-test's	GluN2A synaptic: Cont. *vs* Env. P = 0.680 GluN2B synaptic: Cont. *vs* Env. ***P < 0.0001	Non-gaussian
Figure 1F	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$) (normalized to baseline (pre)-condition for each neuron)	n = 5 (cultures)		Neutral. Ab. Cont., n = 5 Neutral. Ab. Env, n = 5 (neurons)	Neutral. Ab. Cont., n = 261 Neutral. Ab. Env, n = 490 (trajectories)	0.891 ± 0.110 0.931 ± 0.070 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	Neutral. Ab. Cont. *vs* Env, P = 0.178	Non-gaussian
Figure 1G	% Synaptic detections	n = 12-13 (cultures)		Cont., n = 16, Env, n = 17 (neurons)		Dots represent mean synaptic detections (%) from each neuron	n = paired neurons	One tailed, paired t-test's	Cont. pre *vs* post, P = 0.183 Env pre *vs* post, *P = 0.037	Gaussian
Figure 1J	Synaptic cluster area (μm^2) (normalized to control)	n = 4 (cultures)		*GluN2A:* Cont., n = 28 Env, n = 28 *GluN2B:* Cont., n = 35 Env, n = 35 (neurons)	*GluN2A:* Cont., n = 58 Env, n = 66 *GluN2B:* Cont., n = 75 Env, n = 74 (dendrites)	GluN2A: 1.00 ± 0.044 1.166 ± 0.047 GluN2B: 1.00 ± 0.036 1.096 ± 0.045 (mean ± s.e.m.)	n = dendrites	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	*GluN2A:* Cont. vs Env, *P = 0.031 *GluN2B:* Cont. *vs* Env, P = 0.110	Non-gaussian
Figure 2B	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$) (normalized to baseline (pre)-condition for each neuron)	n = 6 (cultures)		Cont., n = 7 Env, n = 6 (neurons)	Cont., n = 295 Env, n = 377 (trajectories)	0.815 ± 0.066 0.889 ± 0.084 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	Cont. *vs* Env, P = 0.099	Non-gaussian
Figure 2B (right)	Synaptic GluN2B mean square displacement, MSD (μm^2), of raw data from 2B	n = 6 (cultures)		Cont., n = 7 Env, n = 6 (neurons)	Cont., = 295 Env, n = 377 (trajectories)					
Figure 2C	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$) (normalized to baseline (pre)-condition for each neuron)	n = 5 (cultures)		Cont., n = 5 Env, n = 6 (neurons)	αTLR-4 + Cont., n = 163 αTLR-4 + Env, n = 276 (trajectories)	1.077 ± 0.107 1.018 ± 0.067 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	Cont. *vs* Env, P = 0.667	Non-gaussian
Figure 2C (right)	Synaptic GluN2B mean square displacement, MSD (μm^2), of raw data from 2C	n = 5 (cultures)		Cont., n = 5 Env, n = 6 (neurons)	αTLR-4 + Cont., n = 163 αTLR-4 + Env, n = 276 (trajectories)					

Main Figures	Parameters	Sample size	Nr. Rep. exp.	Sample collection	Sample collection-Conditions	Values	Statistical (n) value	Statistical test(s)	P-value (alpha = 0.05)	Assumptions and corrections
Figure 2D	Paired mean flourecence intencity (normalised to control)	n = 5 (cultures)	2	Cont., n = 5 Env, n = 5 (cultures)	*TNF-* α: Cont. Env *IL-6* : Cont. Env *IL-1α* : Cont. Env *IL-1β* : Cont. Env *INF- γ* : Cont. Env IL-18: Cont. Env IL-10: Cont. Env	1.000 ± 0.034 1.195 ± 0.094 1.000 ± 0.018 1.136 ± 0.046 1.000 ± 0.027 1.286 ± 0.094 1.000 ± 0.014 1.475 ± 0.257 1.000 ± 0.016 1.199 ± 0.085 1.000 ± 0.037 1.131 ± 0.094 1.000 ± 0.088 1.152 ± 0.125 (mean ± s.e.m.)	n = paired cultures	One tailed, paired t-test	TNF-α:Cont. *vs* Env, **P = 0.0091 IL-6:Cont. *vs* Env, *P = 0.0374 IL-1α:Cont. *vs* Env, **P = 0.0087 IL-1β: Cont. *vs* Env, *P = 0.0264 INF-γ:Cont. *vs* Env, *P = 0.0263 IL-18:Cont. *vs* Env, P = 0.0886 IL-10:Cont. *vs* Env, P = 0.2080	Gaussian
Figure 2E	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$) (normalized to baseline (pre)-condition for each neuron)	n = 4-6 (cultures)		Cont., n = 6 Env, n = 6 αTNF-α + Env, n = 5 αIL-6 + Env, n = 4 IL-1ra + Env, n = 7 (neurons)	Cont., n = 579 Env, n = 352 αTNF-α + Env, n = 461 αIL-6 + Env, n = 404 IL-1ra + Env, n = 365 (trajectories)	0.878 ± 0,049 1.148 ± 0,070 1.010 ± 0,062 0.814 ± 0,045 0.665 ± 0,063 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by Kruskal-Wallis test	Kruskal-Wallis statisitcs = 81.15, ***P <0.0001, Dunn's multiple comparisons test: Cont. *vs* Env, ***P = 0.0006 Cont. *vs* αTNF-α + Env, P > 0.999 Cont. *vs* αIL-6 + Env, P > 0.999 Cont. *vs* IL-1ra + Env, ***P < 0.0001 Env *vs* αTNF-α + Env, P = 0.0861 Env *vs* αIL-6 + Env, P = 0.0711 Env *vs* IL-1ra + Env, ***P < 0.0001	Non-gaussian
Figure 2F	Synaptic GluN2B mean square displacement, MSD (μm^2), of raw data from 2E	n = 4-6 (cultures)		Cont., n = 6 Env, n = 6 IL-1ra + Cont., n = 7 IL-1ra + Env, n = 7 (neurons)	Cont., n = 579 Env, n = 352 IL-1ra + Cont., n = 176 IL-1ra + Env, n = 365 (trajectories)					
Figure 2G	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$) (normalized to baseline (pre)-condition for each neuron)	n = 7 (cultures)		Cont., n = 7 IL-1β (1ng/ml), n = 7 (neurons)	Cont., n = 423 IL-1β (1ng/ml), n = 480 (trajectories)	0.785 ± 0.065 0.147 ± 0.095 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by Two tailed Mann Whitney test	Cont. *vs* IL-1β, ***P < 0.0001	Non-gaussian
Figure 2H	Number of IL-1R clusters / µm (normalized to control)	n = 4 (cultures)		Cont., n = 32 Env, n = 31 (neurons)	Cont., n = 55 Env, n = 51 (dendrites)	1.00 ± 0.194 1.938 ± 0.356 (mean ± s.e.m.)	n = dendrites	D'Agostino & Pearson omnibus normality test followed by Two tailed Mann Whitney test	Cont. *vs* Env, *P = 0.0188	Non-gaussian
Figure 2I	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$) (normalized to baseline (pre)-condition for each neuron)	n = 6 (cultures)		PP2 + Cont., n = 6 PP2 + Env, n = 6 (neurons)	PP2 + Cont., n = 743 PP2 + Env, n = 492 (trajectories)	0.874 ± 0.038 0.702 ± 0.042 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by Two tailed Mann Whitney test	PP2: Cont. *vs* Env, **P = 0.0067	Non-gaussian
Figure 2I (right)	Synaptic GluN2B mean square displacement, MSD (μm^2), of raw data from 2I	n = 6 (cultures)		PP2 + Cont., n = 6 PP2 + Env, n = 6 (neurons)	PP2 + Cont., n = 743 PP2 + Env, n = 492 (trajectories)					
Figure 3B	Ca^{2+} transients frequency (Hz)	n = 3-4 (cultures)		Cont., n = 8 Env, n = 10 Cont. + IL-1ra, n = 7 Env + IL-1ra, n = 7 (neurons)	Cont., n = 88 Env, n = 73 Cont. + IL-1ra, n = 115 Env + IL-1ra, n = 101 (spines)	0.130 ± 0.0052 0.161 ± 0.0071 0.147 ± 0.0042 0.120 ± 0.0050 (mean ± s.e.m.)	n = spines	D'Agostino & Pearson omnibus normality test followed by two-way ANOVA	ANOVA table: Interaction: F(1,373) = 35,68, ***P < 0.0001 Treatment: F(1,373) = 7,195, ** P = 0.0076 Group F(1,373) = 0,733, P = 0.3925 Bonferroni's multiple comparisons test: Env *vs* Cont., ***P < 0.0001 Env *vs* Cont. + IL-1ra, P = 0.0837 Env *vs* Env + IL-1ra, ***P < 0.0001	Data was transformed to gaussian distribution by x=sqrt(x)
Figure 3D	Mean spine Ca^{2+} transients correlation	n = 3-4 (cultures)		Cont., n = 8 Env, n = 10 Cont. + IL-1ra, n =7 Env + IL-1ra, n =7 (neurons)	Cont., n = 88 Env, n = 73 Cont. + IL-1ra, n = 115 Env + IL-1ra, n = 101 (spines)	0.0634 ± 0.0061 0.2142 ± 0.0119 0.1230 ± 0.0090 0.1353 ± 0.0102 (mean ± s.e.m.)	n = spines	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test`s	Cont. *vs* Env, ***P < 0.0001 Cont. + IL-1ra *vs* Env + IL-1ra, P = 0.470	Non-gaussian
Figure 3F	Mean spine Ca^{2+} transients correlation	n = 4-5 (cultures)		E.vector, n = 8 Env, n = 8 E.vector + IL-1ra, n = 8 Env + IL-1ra, n = 8 (neurons)	E.vector, n = 126 Env, n = 159 E.vector + IL-1ra, n = 114 Env + IL-1ra, n = 139 (spines)	0.11421 ± 0.0066 0.2013 ± 0.0067 0.1424 ± 0.0084 0.1141 ± 0.0059 (mean ± s.e.m.)	n = spines	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test`s	E. vector *vs* Env, ***P < 0.0001 E. vector + IL-1ra *vs* Env + IL-1ra, *P = 0.0260	Non-gaussian

Main Figures	Parameters	Sample size	Nr. Rep. exp.	Sample collection	Sample collection-Conditions	Values	Statistical (n) value	Statistical test(s)	P-value (alpha = 0.05)	Assumptions and corrections
Figure 3H	Synaptic GluA1-AMPAR intencity (a.u.) (cumulative distribution)	n = 4 (cultures)		Cont., n = 25 Env, n = 21 (neurons)	Cont., n = 1071 Env, n = 728 (spines)	540 ± 489-653 743 ± 604-1025 (median ± IQR)	n = spines	D'Agostino & Pearson omnibus normality test followed by Kolmogorov-Smirnov test	Cont. *vs* Env, ***P < 0.0001	Non-gaussian
Figure 3J	Ratio Synaptic GluA1-AMPAR intencity (a.u.) (post/pre)	n = 4 (cultures)		noLTP Cont., n = 13 cLTP Cont., n = 12 noLTP Env, n = 17 cLTP Env, n = 14 (neurons)	noLTP Cont., n = 621 cLTP Cont., n = 902 noLTP Env, n = 461 cLTP Env, n = 472 (spines)	0.972 ± 0.922-1.015 0.982 ± 0.928-1.024 0.994 ± 0.956-1.040 0.949 ± 0.904-0.989 (median ± IQR)	n = spines	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test`s	Cont.: noLTP *vs* cLTP, *P = 0.0233 Env: noLTP *vs* cLTP, ***P < 0.0001	Non-gaussian
Figure 4D	Distance travelled (m)	n = 24 (animals)		Cont., n = 12 Env, n = 12 (animals)	Cont. Day 1 Env Day 1 Cont. Day 2 Env Day 2 Cont. Day 3 Env Day 3	40.73 ± 1.74 42.36 ± 2.16 29.44 ± 2.64 29.46 ± 2.14 31.51 ± 2.78 28.00 ± 1.66 (mean ± s.e.m.)	n = animals	Repeated measurment, two-way ANOVA	ANOVA table: Interaction: F(4,64) = 0.2256, P = 0.9231 Day: F(2,64) = 24.11, P*** < 0.0001 Group: F(2,32) = 0.5953, P = 0.5574 Subj. maching: F(32,64) = 2.434, **P = 0.0012	Gaussian
Figure 4E	Distance travelled (m)	n = 22 (animals)		Cont., n = 10 Env, n = 12 (animals)			n = animals	Repeated measurment, two-way ANOVA, post MK-801 injection, uncorrected Fisher's LSD post-hoc analysis	ANOVA table: Interaction: F(23,460) = 0.9119, P = 0.5826 Time: F(23,460) = 22.18, ***P < 0.0001 Group: F(1,20) = 7.778, *P = 0.0113 Subj. maching: F(20,460) = 24.32, ***P < 0.0001. Uncorrected Fisher's LSD post-hoc analysis for each timepoint (5min interval): Significant differences: *P(t80-110 and 125-170) = 0.011-0.039 **P(t135,140 and 150) = 0.0048-0.0079	Gaussian
Figure 4G	Active interaction (sec.)	n = 13 (animals)		Cont., n = 6 Env, n = 7 (animals)		Control Training: 50.67 ± 1.91 Recall: 40.00 ± 4.23 Env Training: 52.71 ± 1.94 Recall: 52.57 ± 2.57 (mean ± s.e.m.)	n = animals	Repeated measurment, two-way ANOVA followed by Sidak's multiple comparisons test	ANOVA table: Interaction: F(1,11) = 7.837, *P = 0.0173 Trial: F(1,11) = 8.269, *P = 0.0151 Group: F(1,11) = 4.579, P = 0.0556 Subj. maching: F(11,11) = 3.303, *P 0.0297. Sidak's multiple comparisons test Cont. Training *vs* Recall: **P = 0.0052 Env Training *vs* Recall P = 0.998	
Figure 4I (left)	% of pre pulse inibition	n = 62 (animals)		Cont., n = 31 Env, n = 31 (animals)	+4dB: Cont. Env +8dB:Cont. Env +12dB: Cont. Env (animals)	40.12 ± 3.036 27.54 ± 3.244 49.41 ± 3.571 33.70 ± 3.300 53.14 ± 2.661 46.00 ± 3.295 (mean ± s.e.m.)	n = animals	Two-way ANOVA, followed by Bonferroni's multiple comparisons test	ANOVA table: Interaction: F(2,180) = 0.9170, P = 0.4016 Prepulse: F(2,180) = 12.12, ***P < 0.0001 Group: F(1,180) = 20.46 ***P < 0.0001 Bonferroni's multiple comparisons test: +4dB Cont. *vs* Env *P = 0.0180 +8dB Cont. *vs* Env **P = 0.0019 +12dB Cont. *vs* Env P = 0.3472	Gaussian
Figure 4I (right)	% of pre pulse inibition	n = 24 (animals)		Cont.+ Clozapine, n=12 Env + Clozapine, n=12 (animals)	+8dB:Cont. Env (animals)	51.52 ± 4.233 49.72 ± 6.955 (mean ± s.e.m.)	n = animals	D'Agostino & Pearson omnibus normality test followed by two tailed t-test	Cont.*vs* Env, P = 0.8278	Gaussian
Figure 4K	% of pre pulse inibition	n = 86 (animals)		Cont., n = 31 Env, n =11-31 (animals)	Cont., n = 31 Env, n = 31 Cont. IgG: Env, n = 13 cross-link: Env, n = 11 (animals)	49.41 ± 3.571 33.70 ± 3.300 30.59 ± 4.575 41.02 ± 6.530 (mean ± s.e.m.)	n = animals	One-way ANOVA, followed by Tukey's multiple comparisons test	ANOVA : F (3,82) = 4.683 Tukey's multiple comparisons test: Cont. *vs* Env, **P = 0.0093 Cont. *vs* Env Cont.IgG, *P = 0.0195 Cont. *vs* Env cross-link, P = 0.5970 Env *vs* Env Cont.IgG, P = 0.9606 Env *vs* Env cross-link, P = 0.6960 Env Cont.IgG *vs* Env cross-link, P = 0.5456	Gaussian, corrected for unweighted means

Main Figures	Parameters	Sample size	Nr. Rep. exp.	Sample collection	Sample collection-Conditions	Values	Statistical (n) value	Statistical test(s)	P-value (alpha = 0.05)	Assumptions and corrections
Figure 5D	Relative protein expression (GluN2A/PSD-95)	n = 33 (animals)	3		P7: Cont., n = 8 Env, n = 9 P65: Cont., n = 8 Env, n = 8 (animals)	0.637 ± 0.0446 0.804 ± 0.0849 1.030 ± 0.0417 0.8986 ± 0.0283 (mean ± s.e.m.)	n = animals	Two-way ANOVA, followed by Bonferroni's multiple comparisons test	ANOVA table: Interaction: F(1,29) = 8.686, **P = 0.0063 Age: F(1,29) = 13.85,***P = 0.0008 Group: F(1,29) = 0.523, P = 0.4753 Bonferroni's multiple comparisons test: P7:Control 2A vs. P7:Env 2A, P = 0.0610 P7:Control 2A vs. P65:Control 2A, ***P = 0.0004 P7:Control 2A vs. P65:Env 2A, *P = 0.0211 P7:Env 2A vs. P65:Control 2A, P = 0.1611 P7:Env 2A vs. P65:Env 2A, P = 0.9442 P65:Control 2A vs. P65:Env 2A, P = 0.4217	Gaussian
Figure 5E	Relative protein expression (GluN2B/PSD-95)	n = 33 (animals)	3		P7: Cont., n = 9 Env, n = 8 P65: Cont., n = 8 Env, n = 8 (animals)	2.168 ± 0.1169 2.449 ± 0.1991 1.145 ± 0.0366 1.051 ± 0.0521 (mean ± s.e.m.)	n = animals	Two-way ANOVA, followed by Bonferroni's multiple comparisons test	ANOVA table: Interaction: F(1,29) = 2.457, P = 0.1278 Age: F(1,29) = 101.9,***P < 0.0001 Group: F(1,29) = 0.609, P = 0.4416 Bonferroni's multiple comparisons test: P7:Control 2B vs. P7:Env 2B, P = 0.6170 P7:Control 2B vs. P65:Control 2B, ***P < 0.0001 P7:Control 2B vs. P65:Env 2B, ***P < 0.0001 P7:Env 2B vs. P65:Control 2B, ***P < 0.0001 P7:Env 2B vs. P65:Env 2B, ***P < 0.0001 P65:Control 2B vs. P65:Env 2B, P > 0.999	Gaussian
Figure 5F	Immunoprecipitation: Relative protein expression (normalized to control)	n = 50 (animals)	2	P7: Cont., n = 7 Env, n = 6 P65: Cont., n = 7 Env, n = 6 (animals)	GluN2A-P7: Cont., n = 6 Env, n = 6 GluN2B-P7: Cont., n = 7 Env, n = 5 GluN2A-P65: Cont., n = 7 Env, n = 6 GluN2B-P65: Cont., n = 7 Env, n = 6 (animals)	1.0 ± 0.1147 1.753 ± 0.3103 1.0 ± 0.1306 1.309 ± 0.0630 1.0 ± 0.1469 1.043 ± 0.2130 1.0 ± 0.0794 0.9497 ± 0.0857 (mean ± s.e.m.)	n = animals	Two tailed unpaired t-test's	GluN2A: P7: Cont. *vs* Env, *P = 0.0462 P65: Cont. *vs* Env, P = 0.0912 GluN2B: P7: Cont. vs Env, P = 0.8680 P65: Cont. vs Env, P = 0.6748	Gaussian
Figure 5G	Relative protein expression (GluA2/PSD-95)	n = 17 (animals)	2		Cont., n = 8 Env, n = 9 (animals)	0.6725 ± 0.0212 0.8136 ± 0.0408 (mean ± s.e.m.)	n = animals	D'Agostino & Pearson omnibus normality test followed by two tailed unpaired t-test	Cont. *vs* Env, **P = 0.0098	Gaussian
Figure 5H	Relative protein expression (PSD-95/total protein)	n = 34 (animals)	3		P7: Cont., n = 9 Env, n = 9 P65: Cont., n = 8 Env, n = 8 (animals)	0.3776 ± 0.0411 0.3647 ± 0.0432 1.582 ± 0.1683 2.102 ± 0.2001 (mean ± s.e.m.)	n = animals	Two tailed unpaired t-test's	P7: Cont. *vs* Env, P = 0.8320 P65: Cont. *vs* Env, P = 0.067	Gaussian
Figure 5J	% of pre pulse inibition	n = 37 (animals)		Cont.+ Neut. Ab, n=13 Env + Cont. Ab, n=12 Env + Neut. Ab, n=12 (animals)	+12dB:Cont.+ Neut. Ab Env + Cont. Env + Neut. Ab (animals)	59.18 ± 3.817 38.98 ± 5.589 58.21 ± 7.049 (mean ± s.e.m.)	n = animals	One-way ANOVA, followed by Tukey's multiple comparisons test	ANOVA : F (3,82) = 4.153, *P = 0.0243 Tukey's multiple comparisons test: Cont. + Neut. Ab *vs* Env + Cont. Ab, *P = 0.0368 Cont. + Neut. Ab *vs* Env + Neut. Ab, P = 0.9915 Env + Cont. Ab *vs* Env + Neut. Ab, P = 0.0542	Gaussian
Suppl. Figures	**Parameters**	**Sample size**	**Nr. Rep. exp.**	**Sample collection**	**Sample collection-Conditions**	**Values**	**Statistical (n) value**	**Statistical test(s)**	**P-value (alpha = 0.05)**	**Assumptions and corrections**
Fig. S1A	Extrasynaptic GluN2A and GluN2B instantaneous diffusion coefficient (μm²/s) (normalized to baseline(pre)-condition for each neuron)	n = 3-13 (cultures)		*GluN2A:* Cont., n = 3, Env, n = 4 *GluN2B:* Cont., n = 10, Env, n = 9 (neurons)	*GluN2A: Extra Syn.:* Cont., n = 300 Env, n = 445 *GluN2B: Extra Syn.:* Cont., n = 819 Env, n = 727 (trajectories)	0.863 ± 0.064 0.941 ± 0.056 0.895 ± 0.045 0.955 ± 0.048 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney t-test's	GluN2A extrasynaptic: Cont. *vs* Env. P = 0.4331 GluN2B extrasynaptic: Cont. *vs* Env. *P = 0.0319	Non-gaussian

Suppl. Figures	Parameters	Sample size	Nr. Rep. exp.	Sample collection	Sample collection-Conditions	Values	Statistical (n) value	Statistical test(s)	P-value (alpha = 0.05)	Assumptions and corrections
Fig. S1B	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$) (normalised to baseline for each neuron)	n = 5-7 (cultures)		Cont., n = 8, Neutral. Ab. Cont., n = 5 Env 0.5µg/ml, n = 7 Env 1.0µg/ml, n = 6 Env 10µg/ml, n = 7 (neurons)	Cont., n = 164 Neutral. Ab. Cont., n = 261 Env 0.5µg/ml, n = 90 Env 1.0µg/ml, n =204 Env 10µg/ml, n = 140 (trajectories)	0,861 ± 0,137 0.891 ± 0.110 0,707 ± 0,168 1,129 ± 0,1252 1,015 ± 0,146 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by Kruskal-Wallis test	Kruskal-Wallis statistic = 40.35, P*** <0.0001 Dunn's multiple comparisons test: Cont. *vs* Env 0.5, P = 0.5142 Cont. *vs* Env 1.0, **P = 0.0015 Cont. *vs* Env 10, P = 0.7632	Non-gaussian, corrected for unweighted means
Fig. S1C	Median GluN2B instantaneous diffusion coefficient ($\mu m^2/s$)	n=12-13 (cultures)		Cont., n= 16 (pre-post), Env, n= 17 (pre-post) (neurons)	Cont., n= 670 Env, n= 792 (trajectories)	Dots represent median diffusion coefficient ($\mu m^2/s$) from each neuron	n = paired neurons	Two tailed, paired t-test's	Cont. pre *vs* post, P = 0.081 Env pre *vs* post, *P = 0.038	Gaussian
Fig. S1D	Synaptic GluN2B mean square displacement, MSD (μm^2), of data in main fig 1E and F	n = 12-13 (cultures)		Cont., n = 16 Env, n = 17 (neurons)	Cont., n = 670 Env, n = 792 (trajectories)					
Fig. S1F	Extra synaptic instantaneous diffusion coefficient ($\mu m^2/s$)	n = 2-7 (cultures)		*GluN2A:* Cont., n = 28 Env, n = 31 *GluN2B:* Cont., n = 65 Env, n = 51 (neuronal fields)	*GluN2A:* Cont., n = 2214 Env, n = 1670 *GluN2B:* Cont., n = 3882 Env, n = 3872 (trajectories)	0.129 ± 0.027-0.247 0.074 ± 0.025-0.270 0.119 ± 0.016-0.244 0.128 ± 0.025-0.250 (median ± IQR)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	*GluN2A:* Cont. *vs* Env, P = 0.0896 *GluN2B:* Cont. *vs* Env, **P = 0.0029	Non-gaussian
Fig. S1G	Synaptic instantaneous diffusion coefficient ($\mu m^2/s$)	n = 2-4 (cultures)		*GluN2A:* Cont., n = 15 Env. 0.5µg/ml, n = 6 Env. 1.0µg/ml, n =13 *GluN2B:* Cont., n = 22 Env. 0.5µg/ml, n = 19 Env. 1.0µg/ml, n = 18 Neutral. Ab. Env, n = 14 (neurons)	*GluN2A:* Cont., n = 382 Env. 0.5µg/ml, n = 147 Env. 1.0µg/ml, n =277 *GluN2B: :* Cont., n = 612 Env. 0.5µg/ml, n = 485 Env. 1.0µg/ml, n = 600 Neutral. Ab. Env, n = 204 (trajectories)	0.079 ± 0.021-0.167 0.062 ± 0.015-0.190 0.081 ± 0.017-0.191 0.074 ± 0.010-0.153 0.049 ± 0.011-0.148 0.094 ± 0.026-0.190 0.048 ± 0.015-0.128 (median ± IQR)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by Kruskal-Wallis test's	*GluN2A:* Kruskal-Wallis statistic = 0.660, P = 0.7190 *GluN2B:* Kruskal-Wallis statistic = 36.44, ***P < 0.0001 Dunn's multiple comparisons test: Cont. *vs* Env 0.5, P = 0.6928 Cont. *vs* Env 1.0, ***P < 0.0001 Env 0.5 *vs* Env 1.0, ***P < 0.0001 Neutr. Ab Env *vs* Env 1.0, ***P < 0.0001	Non-gaussian , corrected for unweighted means
Fig. S1H	Synaptic GluN2B mean square displacement, MSD (μm^2)	n = 4 (cultures)		*GluN2B:* Cont., n = 22 Env. 0.5µg/ml, n = 19 Env. 1.0µg/ml, n = 18 Neutral. Ab. Env, n = 14 (neurons)	*GluN2B: :* Cont., n = 612 Env. 0.5µg/ml, n = 485 Env. 1.0µg/ml, n = 600 Neutral. Ab. Env, n = 204 (trajectories)					
Fig. S1J (right)	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$)	n = 5-7 (cultures)		Heat Inact. Cont., n = 7 Heat Inact. Env, n = 5 (neurons)	Heat Inact. Cont., n = 563 Heat Inact. Env, n = 443 (trajectories)	0.840 ± 0.047 0.915 ± 0.060 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	Heat Inact. Cont. *vs* Env, P = 0.269	Non-gaussian
Fig. S1J (right)	Synaptic GluN2B mean square displacement, MSD (μm^2), of data in J	n = 5-7 (cultures)		Heat Inact. Cont., n = 7 Heat Inact. Env, n = 5 (neurons)	Heat Inact. Cont., n = 563 Heat Inact. Env, n = 443 (trajectories)					
Fig. S1K	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$)	n = 3-4 (cultures)		Cont. + TTX, n = 3 Env + TTX, n = 4 (neurons)	Cont. + TTX, n = 181 Env + TTX, n = 182 (trajectories)	0.675 ± 0.081 1.166 ± 0.129 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	TTX Cont. *vs* Env, ***P = 0.0008	Non-gaussian
Fig. 1K (right)	Synaptic GluN2B mean square displacement, MSD (μm^2), of data in K	n = 3-4 (cultures)		Cont. + TTX, n = 3 Env + TTX, n = 4 (neurons)	Cont. + TTX, n = 181 Env + TTX, n = 182 (trajectories)					
Fig. S2B	TLR-4 staining (% of area)	n = 2 (cultures)		Cont. emptyRNA, n = 10 TLR-4 shRNA, n = 13 (microglia cells)		1.430 ± 0.277 0.701 ± 0.142 (mean ± s.e.m.)	n = microglia cells	Two tailed, unpaired t-test	Cont. *vs* shRNA, *P = 0.0214	Gaussian
Fig. S2C	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$)(normalized to baseline(pre)-condition for each neuron)	n = 4-5 (cultures)		Cont., n = 4 αTLR-4 + Cont, n = 5 (neurons)	Cont., n = 168 αTLR-4 + Cont, n = 163 (trajectories)	1.059 ± 0,1211 1.077 ± 0,107 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	Cont. *vs* Env, P = 0.641	Non-gaussian

Suppl. Figures	Parameters	Sample size	Nr. Rep. exp.	Sample collection	Sample collection-Conditions	Values	Statistical (n) value	Statistical test(s)	P-value (alpha = 0.05)	Assumptions and corrections
Fig. S2D	Synaptic GluN2B instantaneous diffusion coefficient ($\mu m^2/s$)(normalized to baseline(pre)-condition for each neuron)	n = 4-6 (cultures)		Cont., n = 6 LPS 0.01µg/ml, n = 5 LPS 0.1µg/ml, n = 5 LPS 1µg/ml, n = 6 Glia free Cont., n = 4 Glia free LPS 1µg/ml, n = 4 (neurons)	Cont., n = 340 LPS 0.01µg/ml, n = 281 LPS 0.1µg/ml, n =329 LPS 1µg/ml, n = 446 Glia free Cont., n = 547 Glia free LPS 1µg/ml, n = 312 (trajectories)	0.843 ± 0,078 0.797 ± 0,078 0.993 ± 0,102 1.419 ± 0,096 0.892 ± 0,061 0.965 ± 0.090 (mean ± s.e.m.)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by Kruskal-Wallis test or two tailed Mann Whitney test	Kruskal-Wallis statistic = 24.65 ***P < 0.0001 Dunn's multiple comparisons test: Cont. *vs* Env 0.01, P > 0.9999 Cont. *vs* Env 0.1, P > 0.9999 Cont. *vs* Env 1.0, ***P = 0,0004 Glia free: Cont. *vs* Env P = 0.2824	Non-gaussian
Fig. S2F	Microglia Area (μm^2)	n = 2 (cultures)			Cont., n = 1763 Env, n = 1420 LPS, n = 1279 (microglia cells)	404.1 ± 10.55 433.5 ± 13.28 832.5 ± 20.30 (mean ± s.e.m.)	n = microglia cells	D'Agostino & Pearson omnibus normality test followed by Kruskal-Wallis test	Kruskal-Wallis statisitcs = 467.6, ***P < 0.0001 Dunn's multiple comparisons test: Cont. *vs* Env, *** P < 0.0001 Cont. *vs* LPS, ***P < 0.0001 Env *vs* LPS, ***P < 0.0001	Non-gaussian
Fig. S2G	Transformation index (a.u.) (cumulative distribution)	n = 2 (cultures)			Cont., n = 1763 Env, n = 1420 LPS, n = 1279 (microglia cells)	3.113 ± 2.152-5.029 3.042 ± 2.127-4.892 4.177 ± 2.773-6.822 (median ± IQR)	n = microglia cells	D'Agostino & Pearson omnibus normality test followed by Kruskal-Wallis test	Kruskal-Wallis statisitcs = 192.2, ***P < 0.0001 Dunn's multiple comparisons test: Cont. *vs* Env, P > 0.9999 Cont. *vs* LPS, ***P < 0.0001 Env *vs* LPS, ***P < 0.0001	Non-gaussian
Fig. S4B	Instantaneous diffusion coefficient ($\mu m^2/s$)	n = 2 (cultures)		*Dopamine-D1:* Cont., n = 24 Env, n = 24 (neuronal fields)	*Dopamine-D1:* Cont., n = 14211 Env, n = 9129 (trajectories)	0.0422 ± 0.017-0.087 0.0398 ± 0.015-0.084 (median ± IQR)	n = trajectories	D'Agostino & Pearson omnibus normality test followed by two tailed Mann Whitney test	*Dopamine-D1:* Cont. *vs* Env, ***P = < 0.0001	Non-gaussian
Fig. S5C	Tunel positive cells/mm²	n = 6 (animals)	2	Cont., n = 3 Env, n = 3 (animals)	*DG:* Cont. Env *Pyr.:* Cont. Env *SO*: Cont. Env	13.95 ± 3.48 9.16 ± 1.94 23.22 ± 5.60 16.21 ± 6.64 26.21 ± 7.48 27.53 ± 3.42 (mean ± s.e.m.)	n = animals	D'Agostino & Pearson omnibus normality test followed by two-way ANOVA	ANOVA table: Interaction: F(2,12) = 0.3511, P = 0.7109 Area: F(2,12) = 4.429, *P = 0.0363 Group: F(1,12) = 0.6917, P = 0.4218	Gaussian
Fig. S5E	% Iba1 positive cells	n = 17 (animals)		P7: Cont., n = 4 Env, n = 5 P65: Cont., n = 3 Env, n = 5 (animals)	P7: Cont., n = 16 Env, n = 20 P65: Cont., n = 5 Env, n = 14 (hippocampi)	8.265 ± 0.5509 10.94 ± 0.6617 19.40 ± 2.319 16.49± 1.428 (mean ± s.e.m.)	n = hippocampi	Two tailed unpaired t-test's	P7: Cont. *vs* Env, **P = 0.0050 P65: Cont. *vs* Env, P = 0.3070	Gaussian
Fig. S6B	Time spent in the center (% of total time)	n = 32 (animals)		Cont., n = 16 Env, n = 16 (animals)	Cont. Env (animals)	8.78 ± 4.49 9.31 ± 4.87 (mean ± SD)	n = animals	Two tailed t -test	Cont. *vs* Env, P = 0.7533	Gaussian
Fig. S6C	% of pre pulse inibition	n = 25 (animals)		Cont., n = 11 Cont.+ empty vector, n = 14 (animals)	+4dB: Cont. Cont. + E. vector +8dB:Cont. Cont. + E. vector +12dB: Cont. Cont. + E. vector (animals)	35.03 ± 5.498 33.88 ± 3.924 42.98 ± 5.699 49.59 ± 4.043 49.54 ± 6.333 46.29 ± 5.919 (mean ± s.e.m.)	n = animals	Two-way ANOVA	ANOVA table: Interaction: F(2,69) = 0.4878, P = 0.6161 Prepulse: F(2,69) = 3.912, *P = 0.0246 Group: F(1,69) = 0.0292, P = 0.8648	Gaussian
Fig. S6D	% of pre pulse inibition	n = 24 (animals)		Cont. + Clozapine, n = 12 Env + Clozapine, n = 12 (animals)	+4dB: Cont. + Clozapine Env + Clozapine +8dB:Cont. + Clozapine Env + Clozapine +12dB:Cont. + Clozapine Env + Clozapine (animals)	33.18± 5.232 36.65± 6.428 51.52± 4.233 49.72±6.955 57.35± 4.459 56.12±7.096 (mean± s.e.m.)	n = animals	Two-way ANOVA	ANOVA table: Interaction F(2,66) = 0.1224, P = 0.8850 Prepulse F(2,66) = 7.411,**P = 0.0012 Group F(1,66) = 0.0009, P = 0.9754	Gaussian
Fig. S6E	% of pre pulse inibition	n = 24 (animals)		Env + Cont. IgG, n = 11 Env + GluN1, n = 13 (animals)	+4dB: Env + Cont. IgG Env + GluN1 +8dB: Env + Cont. IgG Env + GluN1 +12dB: Env + Cont. IgG Env + GluN1 (animals)	34.26 ± 3.790 31.54 ± 3.802 30.59 ± 4.575 41.02 ± 6.530 46.63 ± 5.738 47.08 ± 6.860 (mean ± s.e.m.)	n = animals	Two-way ANOVA	ANOVA table: Interaction: F(2,66) = 0.8326, P = 0.4377 Prepulse: F(2,66) = 3.858, ***P = 0.026 Group: F(1,66) = 0.3957, P = 0.5315	Gaussian

Suppl. Figures	Parameters	Sample size	Nr. Rep. exp.	Sample collection	Sample collection-Conditions	Values	Statistical (n) value	Statistical test(s)	P-value (alpha = 0.05)	Assumptions and corrections
Fig. S7E	% of pre pulse inibition	n = 24 (animals)		Cont., n = 12 Env, n = 12 (animals)	+4dB: Cont. Env +8dB:Cont. Env +12dB: Cont. Env (animals)	49.08 ± 3.028 30.27 ± 5.516 49.06 ± 4.172 43.04 ± 5.280 63.50 ± 3.631 51.32 ± 3.627 (mean± s.e.m.)	n = animals	Two-way ANOVA, followed by Bonferroni's multiple comparisons test	ANOVA table: Interaction $F(2,66) = 1.10$, $P = 0.33$ Prepulse $F(2,66) = 8.709$,***$P = 0.0004$ Group $F(1,66) = 12.31$, ***$P = 0.0008$ Bonferroni's multiple comparisons test: +4dB Cont. *vs* Env **$P = 0.0088$ +8dB Cont. *vs* Env $P = 0.6946$ +12dB Cont. *vs* Env $P = 0.1415$	Gaussian
Fig. S7F	% of pre pulse inibition	n = 25 (animals)		Cont. IgG, n = 12 Cont. + Neut. Ab, n = 13 (animals)	+4dB: Cont. Cont. + Neut. Ab +8dB:Cont. Cont. + Neut. Ab +12dB: Cont. Cont. + Neut. Ab (animals)	49.08 ± 3.028 38.61 ± 6.501 49.06 ± 4.172 47.91 ± 6.240 63.50 ± 3.631 59.18 ± 3.817 (mean± s.e.m.)	n = animals	Two-way ANOVA	ANOVA table: Interaction: $F(2,69) = 0.4786$, $P = 0.6217$ Prepulse: $F(2,69) = 7.010$, **$P = 0.0017$ Group: $F(1,69) = 1.806$, $P = 0.1833$	Gaussian
Fig. S8B	Expression (O.D.)	Env neg. n = 6 Env pos. n = 7 (patients, individuals)		Env neg. n = 6 Env pos. n = 7 (patients, individuals)		1.000 ± 0.299 2.129 ± 0.955 1.000 ± 0.079 1.414 ± 0.462 1.000 ± 0.144 1.438 ± 0.285 1.000 ± 0.530 0.059 ± 0.059 1.000 ± 1.000 82.29 ± 24.41 1.000 ± 0.267 0.792 ± 0.222 1.000 ± 0.173 1.060 ± 0.209 (mean ± s.e.m.)	n = patients	Two tailed unpaired t-test's	CRP: Env. neg. *vs* Env. pos., P = 0,2957 TNF-α: Env. neg. *vs* Env. pos., P = 0,4088 IL-6: Env. neg. *vs* Env. pos., P = 0,2193 IL-1α: Env. neg. *vs* Env. pos., P = 0,0817 IL-1β: Env. neg. vs Env. pos., *P = 0,0158 INF-γ: Env. neg. vs Env. pos., P = 0,5575 IL-10: Env. neg. vs Env. pos., P = 0,8313	Gaussian, Welch's correction when necessary
Fig. S8E	Cluster Area (µm²)	Env neg. n = 5 Env pos. n = 6 (patients, individuals)	3-4	Env neg., (n = 3-6) Env pos., (n = 3-6) (neurons/patient-sample/experiment)	*GluA1* : Env neg., n = 57 Env pos., n = 43 *GluN1* : Env neg., n = 56 Env pos., n = 61 *D1* : Env neg., n = 54 Env pos., n = 61 *GABA* $_{\gamma 2}$: Env neg., n = 59 Env pos., n = 68 (neurons)	1.000 ± 0.099 0.940 ± 0.114 1.000 ± 0.046 0.886 ± 0.034 1.000 ± 0.070 0.913 ± 0.078 1.000 ± 0.054 0.150 ± 0.073 (mean ± s.e.m.)	n = neurons	D'Agostino & Pearson omnibus normality test followed by Two tailed unpaired t-test's	GluA1: Env. neg. *vs* Env. pos., P = 0.3946 GluN1: Env. neg. *vs* Env. pos., *P = 0.0479 D1: Env. neg. *vs* Env. pos., P = 0.2211 GABAγ2: Env. neg. *vs* Env. pos., P = 0.2457	Non gaussian data was transformed to gaussian distribution before t-test by x=Log(x)

Table S3. Antibodies, Products and Constructs

Antibodies	Application	Epitope (Immunogen)	Host (Formulation)	Clone	Supplier	Validation
GFP Tag (1:500)	ICC, IHC	The GFP was isolated directly from the jellyfish Aequorea victoria	Polyclonal Rabbit		Thermo Fisher Scientific, (A6455, Lot:1736965, 1826342)	ELISA, ICC, IF, IHC (supplier)
GFP Tag (1:1000)	Western Blot	Mixture of two clones (7.1 and 13.1)	Monoclonal Mouse	7.1 / 13.1	Roche (Mouse , (11814460001, Lot:11751700)	Western blot, ion-exchange HPLC analyses (supplier)
GluA2 (1:100)	WES	834-883AA (cytoplasmic C-terminus), L21/32	Polyclonal Rabbit, (Affinity purified on immobilized antigen)		Neuromab, USA (75-002, Lot:455-6JD-81c)	Western blot analysis, IHC, Electronmic. (supplier)
GluN1 (0.8µg/µl)	Intra hippocampal injection	385-399AA (extracellular)	Polyclonal Rabbit, (Affinity purified on immobilized antigen)		Alomone labs, Jerusalem, Israel (AGC-001, Lot:AN0302)	Western blot analysis (supplier)
GluN2A (1:400)	Qdot-experiment	41-53AA (extracellular)	Polyclonal Rabbit		Alomone labs, (AGC-002, Lot:AN0402, AN0502)	Western blot analysis (supplier)
GluN2A (1:200)	WES, IHC	41-53AA (extracellular)	Polyclonal Rabbit		Agrobio, specific custom-made	Western blot, ICC (in lab see Fig. S6)
GluN2B (1:200)	Qdot-experiment	323-337AA (extracellular)	Polyclonal Rabbit		Alomone labs, (AGC-003, Lot:AN1202, AG0540)	Western blot analysis (supplier)
GluN2B (1:250)	WES, IHC	323-337AA (extracellular)	Polyclonal Rabbit		Agrobio, specific custom-made	Western blot , ICC (in lab see Fig. S6)
GN_ENV_01 (block 30µg/ml, staining 1:200)	ELISA, IHC, Neutralization	Targeting an epitope within the ectodomain of MSRV-Env protein	Monoclonal Mouse	13H5/A5	Geneuro,Geneva, Switzerland (GN_01, Lot:060.13144.1)	Western blot , ELISA (supplier)
GN_ENV_03 (block 30µg/ml, neutralizing in vivo 30mg/kg)	Neutralization	Targeting an epitope (different from GN_01) within the ectodomain of MSRV-Env protein	Monoclonal Mouse		Geneuro, (GN_03, Lot:SQ18AK01104 , SQ17AK01201)	Western blot , ELISA (supplier)
GN_Gag_06 (control in vivo 30mg/kg)	Control-Neutralization	Targeting an epitope within the MSRV-Gag protein	Monoclonal Mouse		Geneuro, (GN_03, Lot:SQ18AK01102 , SQ17AK01410)	Western blot , ELISA (supplier)
MsxGlutamine (1:1000)	ICC, IHC	Glutamine synthetase	Monoclonal Mouse IgG2a	GS-6	Merck Millipore, (MAB302, Lot: 2266470)	Western Blot (supplier)
Iba1 (1:1000)	ICC, IHC	Iba1 carboxy-terminal sequence	Polyclonal Rabbit		WAKO, Neuss, Germany (#019-19741, Lot: WEE4506, SAE6921)	ICC, Western Blot (supplier)
IL-1R (1:800)	Western Blot, Immunopersipition	C-terminus of IL-1RI of mouse origin	Polyclonal Rabbit		Santa Cruz, Dallas, Texas, USA (M-20, #sc-	ICC, Western Blot (supplier)
MAP-2 (1:500)	ICC, IHC	Projection domain sequence of recombinant human MAP2, amino acids 377-1505.	Polyclonal Chicken IgY		EnCor Biotechnology Inc.,Gainesville, USA (CPCA-MAP2, Lot:2-1103)	ICC, Western Blot (supplier)
MBP (1:1000)	IHC	Reacts with the sequence Ala-Ser-Asp-Tyr-Lys-Ser in position 131-136 of the classic human myelin basic protein.	Monoclonal Mouse IgG2b kappa	SMI 99	BioLegend Way, San Diego, CA, USA (#808403)	IHC (supplier)
Neutral. IL-6 (1µg/ml)	Neutralization	*E-coli.*-derived recombinant IL-6 Phe25-Thr211, Accession # P20607	Polyclonal Goat		R&D Systems (AF506, Lot: BC20615101)	ICC, Western Blot, Neutralization (supplier)
Neutral. TNF-α (1µg/ml)	Neutralization	*E-coli.*-derived recombinant TNF-alpha Leu80-leu235	Polyclonal Goat		R&D Systems (AF-510-NA, Lot: X10715081)	ICC, Western Blot, Neutralization (supplier)
PSD-95 (1:250)	IHC	Purified recombinant rat PSD-95	Monoclonal Rabbit	7E3-1B8	Thermo Fisher Scientific, (#MA1-046)	Thermo scientific Cellomics High Content Screening kit (supplier)

Antibodies	Application	Epitope (Immunogen)	Host (Formulation)	Clone	Supplier	Validation
PSD-95 (1:50)	WES	Synthetic peptide corresponding to residues of human PSD95	Polyclonal Rabbit		Cell signaling, Danvers, USA, (#2507, Lot: 2)	Thermo scientific Cellomics High Content Screening kit (supplier)
Rabbit IgG (0.8µg/µl)	Intra hippocampal injection	Gamma Immunoglobins Heavy and Light chains	Polyclonal Goat IgG, highly cross-absorbed		Novex, (A16112, Lot:36-96-032615)	
TLR-4 (block 20µg/ml, staining 1:100)	ICC, Neutralization	Anti-human CD284 (TLR4)	Monoclonal Mouse IgG2a, kappa (Functional grade purified)	HTA125	Affymetrix,Wien, Austria (#16-9917-82, Lot:E06988-1633)	Flow cytometric analysis of normal human peripheral blood cells (supplier), ICC (in lab)

Secondary Antibodies	Application	Epitope (Immunogen)	Host (Formulation)	Clone	Supplier	Validation
Alexa Fluor® 488 (1:500)	ICC, IHC	Gamma Immunoglobins Heavy and Light chains from Rabbit	Polyclonal Goat		ThermoFisher, (A11008, Lot:982425)	Flow Cytometry, ICC, IF, IHC, Certificate of analysis of absorption/ fluorecence and ICC provided (supplier)
Alexa Fluor® 488 (1:500)	ICC, IHC	Gamma Immunoglobins Heavy and Light chains from Mouse	Polyclonal Goat		ThermoFisher, (A11001, Lot:1664729)	Flow Cytometry, ICC, IF, IHC, Certificate of analysis of absorption/ fluorecence and ICC provided (supplier)
Alexa Fluor® 568 (1:500)	ICC, IHC	Gamma Immunoglobins Heavy and Light chains from Rabbit	Polyclonal Goat		ThermoFisher, (A11011, Lot:1670154)	Flow Cytometry, ICC, IF, IHC, Certificate of analysis of absorption/ fluorecence and ICC provided (supplier)
Alexa Fluor® 568 (1:500)	ICC, IHC	Gamma Immunoglobins Heavy and Light chains from Mouse	Polyclonal Goat		ThermoFisher, (A11004, Lot:927620)	Flow Cytometry, ICC, IF, IHC, Certificate of analysis of absorption/ fluorecence and ICC provided (supplier)
Alexa Fluor® 647 (1:500)	ICC, IHC	Gamma Immunoglobins Heavy and Light chains from Mouse	Polyclonal Donkey		ThermoFisher, (A37571, Lot:1252811)	Flow Cytometry, ICC, IF, IHC, Certificate of analysis of absorption/ fluorecence and ICC provided (supplier)
Alexa Fluor® 647 (1:500)	ICC, IHC	Gamma Immunoglobins Heavy and Light chains from Chicken	Polyclonal Goat		ThermoFisher, (A21449)	Flow Cytometry, ICC, IF, IHC, Certificate of analysis of absorption/ fluorecence and ICC provided (supplier)
Peroxidase AffiniPure Donkey Anti-Mouse IgG (H+L) (1:10 000)	Western Blot	Mouse IgG (H+L)	Polyclonal Donkey		Jackson ImmunoResearch (#715-035-150, Lot: 129117)	Immuno-electrophoresis and/or ELISA provided (supplier)
Qdot® 655, F(ab')2 Fragment (1:10000)	Qdot-experiment	Gamma Immunoglobins Heavy and Light chains from Rabbit	Polyclonal Goat		ThermoFisher, (Q11422MP)	IF (supplier), Groc et al. 2006, Specificity was checked for each batch on a routine basis (live ICC in lab)

Product	Distributer		Protein	Construct	Tagg	Provider
Bicuculline	Tocris (#0131, Batch:34)		**Dopamine-receptor**	D1-CFP	Cyan Fluorescent Protein (CFP)	Gift from D. Choquet (IINS, Bordeaux)
Clozapine	Abcam (ab120019, Lot:APN10083-1-1)		**Empty vector**	pcDNA3		In Lab
D-AP5	Tocris (#0106, Batch:73)		**GABA-γ^2**	Gabaar y2 phluorin	Super Ecliptic pHluorin (SEP)	Gift from S.Levi (Kittlerprims lab)
Envelope-protein	PXTherapeutics (Grenoble, France)		**GCamP3**	N1-LcK-GCaMP3		Gift from Khakh Lab (UCLA, USA)
Glycine	Euromedex (26-128-6405-C, Lot: 1063521/165246)		**GCamP6**	pZac2.1 GfaABC1D Lck-GCaMP6f		Gift from Khakh Lab
IL-1β	R&D Systems (501-RL, Lot: QZ2514101)		**GFP**	pEGFP-C1		Clontech, California, USA

Product	Distributer
IL-ra	R&D Systems (480-RM, Lot: U11014081, U11815071)
LPS (serotype O26:B6)	Sigma, (L5543, Lot:123M4052V)
(+)-MK 801 maleate	Tocris (#0924, Batch:10)
NBQX	Tocris (#0373, Batch:14)
Nifedipine	Tocris (#1075, Batch:2)
Picrotoxin	Tocris (#1128, Batch:8)
PP2	Calbiochem (#529576)
Tetrodotoxin	Tocris (#1078, Batch:47)
TUNEL-kit	Promega (#G7362+1, Lot: 0000222644+0000223921)

Protein	Construct	Tagg	Provider
GluA1	HA SEP GluA1(pRK5)	SEP	Gift from D. Choquet
GluN1	GluN1-SEP, N-Term (JN)	SEP	Gift from D. Choquet
Homer	pcDNA3.1 - Homer1c	DsRed dimeric	In Lab
MSRV-Env	phCMV-MSRV Env (clone pV14, AF 331500)		Geneuro,Geneva, Switzerland
shTLR-4	Small-hairpin RNA (shRNA)-encoding TLR-4 supression vector		Gift from P. Küry (Heinrich-Heine-University, Düsseldorf)