## SUPPLEMENTARY METHODS

### *EAE Induction*

EAE was induced in C57BL/6 female mice by subcutaneous immunization with 300 µl of 200 µg MOG35−55 (Espikem, Florence) in incomplete Freund's adjuvant supplemented with 8 mg $ml^{-1}$ *Mycobacterium tuberculosis* (strain H37Ra; Difco). Pertussis toxin (Sigma) (500 ng) was injected on the day of the immunization and again two days later. EAE mice were compared with two control (HC) groups, one composed of naïve, untreated mice, and a second of mice treated with complete Freund's adjuvant without MOG, and pertussis toxin.

All efforts were made to minimize animal suffering and to reduce the number of mice used, in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). All procedures involving animals were performed according to the guidelines of the Institutional Animal Care and Use Committee of the San Raffaele Scientific Institute and of the University of Rome Tor Vergata.

### *Electrophysiology*

In both control and EAE brains, the striatum could be readily identified under low power magnification, whereas individual neurons were visualized in situ using a differential interference contrast (Nomarski) optical system. This employed an Olympus BX50WI (Japan) non-inverted microscope with x40 water immersion objective combined with an infra-red filter, a monochrome CCD camera (COHU 4912), and a PC compatible system for analysis of images and contrast enhancement (WinVision 2000, Delta Sistemi, Italy). Recording pipettes were advanced towards individual striatal cells in the slice under positive pressure and, on contact, tight GΩ seals were made by applying negative pressure. The membrane patch was then ruptured by suction and membrane current and potential monitored using an Axopatch 1D patch clamp amplifier (Axon Instruments, Foster City, CA, USA). Whole-cell access resistances measured in voltage clamp were in the range of 5-20 MΩ.

Synaptic events were stored by using P-CLAMP 9 (Axon Instruments) and analyzed off line on a personal computer with Mini Analysis 5.1 (Synaptosoft, Leonia, NJ, USA) software. The detection threshold of spontaneous and miniature excitatory and inhibitory events was set at twice the baseline noise. The fact that no false events would be identified was confirmed by visual inspection for each experiment. Offline analysis was performed on spontaneous and miniature synaptic events recorded during fixed time epochs (3-5 min, 3-6 samplings), sampled every 5 or 10 minutes. Only cells that exhibited stable frequencies (less than 20% changes during the control samplings) were taken into account. For kinetic analysis, events with peak amplitude between 10 and 50 pA were grouped, aligned by half-rise time, and normalized by peak amplitude. Events with complex peaks were eliminated. In each cell, all events between 10 and 50 pA were averaged to obtain rise times, decay times, and half widths (Centonze et al., 2005).

One to six cells per animal were recorded. For each type of experiment and time point, at least four distinct animals were employed for each experimental group. Throughout the text "n" refers to the number of cells, unless otherwise specified. Data are presented as the mean ± S.E.M. Multiple comparisons were analyzed by one-way ANOVA followed by Tukey HSD. Comparisons between two groups were analyzed by paired or unpaired Student's *t*-test or Wilcoxon's test. The significance level was established at $p<0.05$. To determine whether two cumulative distributions of spontaneous synaptic activity were significantly different, the Kolmogorov-Smirnov (K-S test) was used. Correlation analysis was estimated by Pearson test. Drugs were applied by dissolving them to the desired final concentration in the bathing ACSF. Drugs were (in μM): CNQX (10), MK-801 (30), NBQX (10), tetrodotoxin (TTX, 1) (from Tocris Cookson, Bristol, UK). Bicuculline (10) (from Sigma-RBI, St. Louis, USA). TNF-α (0.6) (from Peprotech, Rocky Hill, NJ). TNFR-Ig (1 μL/ml) (courtesy of Angelo Corti).

***GluR1 and GluR1 pSer845 detection in postsynaptic membranes***

The protein composition of the TIF preparation was carefully tested for the absence of presynaptic markers (data not shown). A similar protein composition was obtained in TIF purified from hippocampi and striata of all mice. The protein content was determined using the Bradford protein assay (Bio-Rad, Hercules, CA) and the same amount was applied to SDS-PAGE and electroblotted for all samples. Western blots were performed on polyvinylidene difluoride membranes (PVDF, Immobilon P, Millipore, Billerica, MA). GluR1 was detected with a rabbit polyclonal anti-GluR1 antibody (clone CH3, Upstate, Lake Placid, NY) diluted 1:5000; GluR1 pSer845 was detected with a rabbit polyclonal anti-GluR1 phosphoSer845 antibody (Chemicon International) diluted 1:1000.
All experiments were performed at least twice, unless otherwise indicated. Data are expressed as the means ± S.D. and significance was assessed by Student's *t* test. Differences with $p$ values $< 0.01$ were considered as significant.

***Western blot analysis on whole striatum dissections***

Three HC and three EAE mice per time points were killed at each time point by anaesthetic overdose and transcardially perfused with saline solution. Brains were rapidly removed from the skull and the whole striatum was carefully dissected. Micro-dissections were performed starting from the anterior bregma 1.5 to the posterior bregma -0.5. Homogenate of striatum was obtained by using the following solution: Sucrose 260mM (Sigma); Tris-HCl pH8 10 mM (Sigma); DNaseI (Sigma) 1mg/ml and protease inhibitors (Sigma). Total proteins were quantified by using BCA kit (Pierce) and 20 μg of each sample was applied to a SDS-PAGE and Western blotting was performed as described. The blots were incubated overnight at +4°C with the following antibodies: rabbit α-GluR1 1:5000 (Millipore); rabbit α-GluR1-p845 1:5000(Millipore); goat α-PSD95 (Abcam) 1:3000mouse; rabbit α-Synaptophysin 1:10000 (a gift of Dr. Valtorta F., HSR hospital, Milan-Italy) α-TuJ11:1000(Covance) and α-βActin 1:10000(Sigma); followed by secondary antibodies coupled with HRP (Biorad). The immunoreactivity was visualized by using the ECL detection system (Millipore). Gel images were acquired using a Molecular Dynamics Personal

Densitometer (Amersham Biosciences) and processed by using the ImageQuant software (Amersham Biosciences).

***Flow cytometry in crude synaptosomal preparation (P-2 fraction)***

Annexin-V-phucoerythrin (PE) was purchased from Pharmingen (BD Pharmingen™ 556421 and calcein acetoxymethylester (calcein AM) was obtained from Molecular Probes (Molecular Probes C3100MP).

Mice were killed by cervical dislocation, and the brains were rapidly removed to prepare the P-2 fraction. The striata were pooled (two mice for each experimental condition), and homogenized in 3.6 ml of homogenization buffer (0.32 M sucrose, 4 mM Hepes pH 7.4, 1 mM EGTA, 1 mM PMSF with protease inhibitors). The homogenate was spun at 1,000 g at 4°C for 10 min to remove nuclei and cell debris. The resulting supernatant was centrifuged at 12,000 g at 4°C for 15 min. The pellet was resuspended in 2.5 ml of homogenization buffer and centrifuged (15 min, 13,000 g, 4°C) to obtain the final P-2 fraction. The P-2 fraction was resuspended in binding buffer (10 mM Hepes, pH 7.4, 150 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1.8 mM $CaCl_2$). For dye labelling, dye diluted in binding buffer was added to 0.1 ml aliquots of P-2, incubated for 10 min at RT, then diluted in PBS (final volume 0.5 ml) for immediate flow cytometry analysis. For Calcein AM, final dye concentration was 100 nM, and for PE-Annexin V final concentration was 1 mg/ml.

***Preparation and activation of BV2 microglia cell line***

BV2 cells were constructed by infecting primary microglia with a v-raf/v-myc oncogene-carrying retrovirus (J2) (Blasi et al., 1990). The murine BV2 microglia were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, and were maintained in a humidified incubator with 5% $CO_2$. Cells were infected with CMV-GFP retroviral vector (provided by Montini E., San Raffaele Hospital, Milan, Italy) (Montini et al., 2006). GFP positive BV2 cells were treated with the pro-inflammatory cytokines mix (Th1) for 24 hours. Then,

$10^5$ to $10^6$ cytokine-treated or sham-treated cells were placed onto a single slice and incubated for 30-60 min. Fluorescent microscope was used to detect the presence of GFP positive cells within striatum and whole-cell patch clamp recordings were made as above.

***Preparation and activation of primary microglia cell line***

Primary microglia cultures were obtained from C57Bl/6 mice (P0-P2). In brief, meninges were removed from the forebrain and tissue was minced with scissors in cold KRB medium 1x (Menendez Iglesias et al., 1997) containing Albumin 0.3% (Sigma) and MgCl2 0.04% (Sigma). Cortices were incubated in 10 ml of complete KRB medium supplemented with Trypsin 0.25mg/ml at 37°C for 15 min. Then, 10 ml of complete KRB medium supplemented with DNase I 0.05 mg/ml (Sigma) and SB Trypsin inhibitor 0.08 mg/ml (Sigma) to stop the reaction. Then, cell suspension was washed in glial culture medium DMEM (Gibco) supplemented with 10% FBS (Gibco), L-glutamine (1mM) (Gibco), penicillin (100U/ml) (Gibco) and streptomycin (100mg/ml)(Gibco) and $3x10^6$cells/flask were plated in the same medium in a humidified incubator with 5% $CO_2$ in poly lysine treated 75 cm2 Corning tissue flasks. Medium was changed after 3 days in culture. Microglial cells were shaken off the glial mixed cell culture after 12 days.

***Preparation of primary neuronal cell line***

A single pregnant CD1, (n=5) female was killed by decapitation for each cell preparation. E16.5 embryos were dissected in cold HBSS (Gibco)/Glucose 0.6% (Sigma). After removal of the meninges from the whole brain, the two telencephalic vesicles were carefully removed. The isolated vesicles were collected in separate Petri dishes and triturated by using a surgical scalpel. Telencephalic neurons were gently triturated 6-10 fold with a fire-polished Pasteur pipette within the following culture media: F12/DMEM (Gibco); Hepes 4mM pH7.4 (Sigma); Glucose 0.6% (Sigma); Glutamine 2mM (Gibco); supplement N2 (Invitrogen); antibiotics absent. Thereafter, cells were centrifuged at 200Xg for 4 min at room temperature. Then, cells were suspended in culture

media and 1X106 cells were seeded onto 35mm poly-L-lysine coated Petri dishes (Falcon) previously primed for 1hour with the following media: F12/DMEM (Gibco); FCS 7.5% (Gibco) at +37°C. Twelve days later neurons were treated with 100U/ml IL-1β (Euroclone, Pero, Italy), 200U/ml TNF-α (Peprotech, Rocky Hill, NJ), 500U/ml IFN-γ (Becton Dickinson), (Th1 mix) for respectively: 3h,6h and 24 hours (12 hours for *Arc/Arg 3.1* measures). Then, Th1 treated neurons and sham treated neurons were collected and the total RNA extracted as above. *Arc/Arg3.1* mRNA were quantified by using the LightCycler® 480 System (Roche) as above and the *class beta-III Tubulin* gene was used as housekeeping gene (beta III tub. F: 5' CTATGGGGACCTCAACC ACCTTGTG 3'; beta III tub. R: 5' GGTCACAGGCAGCCATCAT GTTC3'). cDNA obtained form 24h Th1 treated neurons was used to amplify both CD119 and p55 receptors by using the following primers: CD119 f5' GGTGACGGGAGCACCTGTTACACATTC3' CD119 r5' CTGTCATCATGGAAAGGAGGGATACAG3'; p55 f5' GTGCTCCTGGCTCTGCTGATGGG G3' p55 r 5' CACTCCCTG CAGACTGTATCCCGCCC3'. Histogram showed the mean value ± S.E.M. of five independent experiments. Statistical analysis was performed using unpaired Student's *t*-test. Neurons were also used to measure GluR1, GluR1pSer485 AMPAr subunits and PSD-95. Briefly, cells were suspended into the following buffer: Sucrose 260 mM (Sigma); Tris-HCl pH8 10 mM (Sigma); DNaseI (Sigma) 1mg/ml and protease inhibitors (Sigma). Then, the total protein extracts were quantified by using BCA kit (Pierce) and 20μg of each sample was used for a Western blot experiment. The blots were incubated overnight at +4°C with goat α-PSD-95 1:3000 followed by secondary antibody coupled with HRP (Biorad). The immunoreactivity was visualized by using the ECL detection system (Millipore). Densitometry was obtained as described. Procedures for staining surface PSD-95. After treatments neurons were fixed in 4% Paraformaldehyde, 4% Sucrose. Then, blocked with PBS, 10% FBS, 0.1% Triton and a goat antibody against PSD-95 (Abcam) was applied at a dilution of 1:800 overnight at +4°C. After thorough washing with PBS, a donkey anti goat secondary antibody (Alexa flour 488) was applied at a dilution of 1:1000. Images were detected by using a 100x objective mounted on an Olympus

BX51 microscopy, digitized with a CCD camera (Leica) and analyzed with Image-J software (supplemented with the gran-filter plug-in). Each experiment was repeated three times with different culture preparation. Statistical significance was determined using Student's t test. Errors in the histogram represent S.E.M.

### *Golgi staining*

Mice were anaesthetized with chloral hydrate deep anaesthesia (400 mg/kg i.p.), perfused through the ascending aorta with a solution of NaCl 0.9 % for 5 minutes, and then followed by 3 % paraformaldehyde with 0.4 % glutaraldehyde in 0.1 M phosphate buffer (PB) pH 7.4. Sections from animals perfused with glutaraldehyde were treated with sodium borohydrate (Sigma, Italy) 0.1 % in PBS 0.01 M pH 7.4. Brains were removed from the skulls and coronal sections cut on a vibrating microtome and collected in phosphate buffered (PB) 0.1 M pH 7.4 at 4°C for subsequent section-Golgi impregnation. Sections were post-fixed in 1 % osmium tetroxide for 20 min, and free floated in 3.5 % aqueous solution of potassium dichromate for 12 h. Then, each section was placed flat on a microscope slide and excess of potassium dichromate was blotted from the edge of the tissue section. Each section was sandwiched with another microscope slide, and the entire assembly was placed in 1-2 % of silver nitrate solution overnight. The microscope slide was then carefully separated and the Golgi-impregnated section were dehydrated, softened with cedar wood oil and mounted under coverglass. The spine density of striatal neurons was calculated in sections from HC (n = 85 dendrites), EAE (n = 84 dendrites), and EAE mice receiving chronic NBQX (n = 87 dendrites) by counting the total number of spines divided by the dendritic length, by using a 40X oil immersion lens. Only completely impregnated dendrites found entirely within the tissue section were used for spine counts.

## References

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