Supporting Information

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SI Experimental Procedures

DNA Constructs. To make the GluR1-UTR-TAC-GFP fusion construct, the nucleotides corresponding to the rat GluR1 5′ UTR (Accession no. NM_031608) and mouse GluR1 3′ UTR (2,600 nt; Accession no. NM_008165) were amplified from rat or mouse hippocampal cDNA. Full-length human interleukin-2 receptor-α (TAC; Accession no. NM_000417), lacking its stop codon, was amplified from pXS-TAC (a generous gift from Robert Wenthold, National Institutes of Health, Bethesda, MD) and inserted in-frame between the NheI/EcoRI restriction sites of pEGFP-N1 (Clontech). The GluR1 5′ UTR fragment and 3′ UTR fragments were then inserted into pCI-Neo (Promega) flanking the TAC-GFP coding region.

Antibodies. The following mouse monoclonal primary antibodies were used in this study: actin (Chemicon), FMRP (Chemicon), GluR2 (Chemicon), MAP2 (Abcam), and RARα (Chemicon). The following rabbit polyclonal primary antibodies were used: GluR1 (Oncogene–Calbiochem), GluR1 (Upstate), GluR2 (Chemicon), RARα (Santa Cruz Biotechnology). The following secondary antibodies from Jackson ImmunoResearch were used: anti-mouse Cy2, anti-rabbit Cy2, anti-mouse Cy3, and anti-rabbit Cy3. The secondary antibodies for electron microscopy were used: anti-mouse Au5 and anti-rabbit Au15 (Ted Pella).

Drugs and Chemicals. The following drugs and chemicals were purchased from Sigma–Aldrich: *all-trans* retinoic acid, anisomycin, actinomycin D, and cycloheximide. Tetrodotoxin was purchased from Tocris Biosciences and D-APV from Fisher.

Cell Cultures, Transfection, Drug Treatment, and Immunolabeling. Primary hippocampal cultures were prepared from the brains of rats at embryonic day 22 and maintained in serum-free Neurobasal medium supplemented with B-27 and Glutamax (GIBCO-BRL) for 2 weeks *in vitro* (32). Human embryonic kidney (HEK)-293 cells were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% (GIBCO-BRL). Neurons were transfected using Lipofectamine 2000 (Invitrogen) with a protocol described previously (32). HEK293 cells were transfected by using HEKfectin (Bio-Rad) according to the manufacturer's instructions. Stock solutions of *all-trans* RA in DMSO were freshly made right before treatment, and the final concentration of DMSO in culture media is 0.05% or lower. Twenty-four-hour treatment of 1 μM TTX and 100 μM APV was used to induce synaptic scaling in dissociated cultures.

Surface Biotinylation Assay. Cultured hippocampal cells were washed four times with cold PBS/Mg^{2+}/Ca^{2+}, and surface proteins were biotinylated with 1 mg/ml Ez-link sulfo-NHS-SS-biotin (Pierce) in ice-cold PBS/Ca^{2+}/Mg^{2+} for 20 min at 4°C. Cells were washed with 0.1 M glycine in ice cold PBS/Ca^{2+}/Mg^{2+} to stop further biotinylation of the surface protein. After four more washes with ice-cold PBS, cells were collected by using centrifugation. Biotinylated cells were solubilized with lysis buffer (PBS with 1% Triton-X, 1% Nonidet P-40, 10% glycerol, 25 mM $MgCl_2$, and a protease inhibitor mixture). Lysates were centrifuged to remove cell debris and nuclei at 20,817 $\times$ *g* for 30 min. Precleared lysate was bound overnight at 4°C using Ultralink-immobilized streptavidin beads to precipitate biotinylated proteins. Nonbiotinylated proteins were removed by centrifugation at 1500 $\times$ *g* for 3 min, and the beads were washed three times with lysis buffer. Biotinylated surface proteins were eluted with denaturing buffer at 75°C. Surface-expressed AMPA receptors were detected by Western blot analysis.

Immunocytochemistry in Slice. Hippocampal slices (400-μm thickness) were fixed in 4% paraformaldehyde for 1 h, rinsed three times with PBS, permeabilized with 0.5% Triton-X 100 for 1 h, and rinsed again three times with PBS. Slices were then treated with 50 mM NH_4Cl for 20 min at room temperature, rinsed three times with PBS and blocked in 10% normal goat serum for 1 h. Slices were subsequently incubated with RARα and MAP2 antibodies for 18 h at 4°C, followed by three 1-h washes in PBS. Cy2-conjugated goat anti-mouse and Cy3-conjugated goat anti-rabbit secondary antibodies were then applied for 18 h at 4°C, followed by five 1-hour PBS washes.

Synaptoneurosome Preparation. Hippocampi from P15-P21 Sprague–Dawley rats were dissected and gently homogenized in a solution containing 33% sucrose, 10 mM Hepes, 0.5 mM EGTA (pH 7.4), and protease inhibitors. Nuclei and other debris were pelleted at 2,000 $\times$ *g* for 5 min at 4°C and the supernatant filtered through three layers of 100-μm pore nylon mesh (Millipore), and a 5-μm pore PVDF syringe filter (Millipore). The filtrate was then centrifuged for 10 min at 10,000 $\times$ *g* at 4°C and the supernatant removed. The synaptoneurosome-containing pellet was then resuspended in the appropriate amount of Minimum Essential Media (Invitrogen) containing protease and RNAsin (Amersham). Equal volumes were then aliquoted into opaque microfuge tubes. Appropriate samples were pretreated for 30 min at 37°C with 50 μM actinomycin D (Sigma), 100 μM cycloheximide (Sigma) or 40 μM anisomycin (Sigma). Appropriate concentrations (100 nM, 1 μM, or 10 μM) of retinoic acid (Sigma) or AM580 (Biomol) were added to the samples, which were incubated for 10 min at 37°C and immediately frozen in dry ice afterward.

***In Situ* Hybridization.** Twenty-four hours after the transfection, cells were fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 for 5 min and incubated in DEPC PBS containing 0.1% active DEPC for 15 min. Cells were then prehybridized with Rapid-Hyb buffer (Amersham Biosciences) in 50% formamide containing 0.1% Blocking Reagent (Roche) at 54°C. Hybridizations were performed at 54°C, and washes at 50°C in 1$\times$ SSC containing 50% formamide. Cells were coincubated with an HRP-linked DIG antibody and rabbit polyclonal MAP2 antibody for 1 h at room temperature and then processed for tyramide signal amplification by using a Cy3-TSA kit (PerkinElmer). MAP2 was detected using a Cy2-conjugated goat anti-rabbit antibody. Digoxygenin-labeled GFP riboprobes recognizing base pairs of 798-1195 in the GFP coding region were transcribed from PCR product T3 and T7 RNA polymerase site adapters.

ImmunoGold EM. Cultured hippocampal neurons were fixed for ImmunoGold electron microscopy for 1 h by using 1% paraformaldehyde (PFA), 2.5% glutaraldehyde (glu), 0.1% tannic acid, and 0.1% picric acid in PBS.

The fixative was washed with PBS, and the cells were incubated in 1% tannic acid on ice for 30 min. This was followed by 2 h of incubation at room temperature in 1% uranyl acetate, and dehydration in ascending concentrations of ethanol. Fixed cells were infiltrated and embedded in LRwhite (Ted Pella).

Sections of 70-nm thickness were cut by using a Reichert–Jung (Leica) ultra-cut microtome. Sections were picked up on 300-mesh nickel grids. To quench free aldehyde groups, grids bearing ultrathin sections were incubated in 1% sodium borohydride for 30 min at room temperature. The sections were then incubated in 5% normal goat serum (NGS) for 1 h and incubated in a mixture of two primary antibodies for 30 min at 37°C. Sections were rinsed in NGS for 15 min before being transferred to a mixture of goat anti-rabbit 15-nm gold-conjugated secondary antibody and goat anti-mouse 5-nm gold-conjugated antibody for 30 min at 37°C. Sections were then washed and counterstained with 1% uranyl acetate and lead citrate and viewed with a JOEL 1200EX transmission electron microscope. RNA granule-like structures in the dendritic regions of neurons were searched for at low magnification because it is easier to distinguish soma versus dendrite at low magnification. After a potential RNA granule was located, we then switched to high magnification to examine whether FMRP labeling was present in the RNA granule-like structure. Almost all of the RNA granule-like structure found at low magnification contained specific FMRP labeling, whereas their labeling with GluR1 or RARα depended on experimental manipulations. Images of all FMRP-positive RNA granules were taken on Kodak 4489 films, and analysis was done by the experimenter who was blind to the experimental conditions of the samples.

Image Acquisition and Quantification. Fluorescent images were acquired at room temperature with an Olympus FV1000 BX61WI laser scanning confocal microscope, using an Olympus Plan Apochromat magnification ×60 oil objective (N.A. 1.42, WD 0.15), an Olympus U-Plan Apochromat magnification ×100 oil objective (N.A. 1.40, WD 0.12), or an Olympus LUMFI magnification ×60 water objective (N.A. 1.10) with sequential acquisition setting at 1,024 × 1,024-pixel resolutions. Laser power and photomultipliers were set such that no detectable bleed-through occurred between different channels. Digital images of the cells were captured with FV1000 Imaging software (Olympus). Eight to 10 sections were taken from top to bottom of the specimen, and brightest-point projections were made. Images for the same experiments were taken by using identical settings for laser power, photomultiplier gain, and offset. These settings were chosen such that the pixel intensities for the brightest samples were just below saturation. Image quantification was performed by experienced investigators who were ''blind'' to the experimental conditions.

Statistical Analysis. Single-factor ANOVA was used for statistical analysis. Values are presented as mean ± SEM in the figures.