Supporting Information

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SI Methods

Cell Culture. Dissociated cultures of hippocampal CA1-CA3 neurons were prepared from P0 rats. Coverslips were coated by spraying 4 mM acetic acid solution containing 5 μg/ml poly-D-lysine (BD Biosciences) and 0.4 mg/ml rat tail collagen. Astrocytes were preplated at 3–6 $\times$ 10^3 per well with culture media consisting of BME (Invitrogen), 10% FCS (BD Biosciences), 1$\times$ Glutamax (Invitrogen), 20 mM D-glucose, and penicillin/streptomycin (BD Biosciences). After 3–7 days, dissociated neurons were plated on astrocytes at 1–2 $\times$ 10^4 per well. At 1 or 2 days *in vitro* (DIV), media were exchanged to 1:3 mixture of culture media (above) and Neurobasal medium (Invitrogen) containing B-27 (Invitrogen), 0.5 mM glutamine, along with 4 μM cytosine-arabinoside (Sigma). In some experiments, cells were treated with TTX (1 μM), CNQX (10 μM), and APV (50 μM) or KCl (5 mM) and bicuculline (10 μM) from 2 days before FM1-43 experiments. Note that BME contained $\approx$5 mM KCl. TTX, CNQX, and APV were purchased from Tocris. Neurons were used for imaging experiments at 14 to 15 and at 20 to 22 DIV. Animal care and use were approved by the U.K. Home Office.

Live Labeling of GluR2. Neurons were first treated with culture medium containing 5 μg/ml anti-GluR2 monoclonal antibody (MAB397, Chemicon) for 15 min, followed by a brief wash with culture medium. Neurons were then treated with culture medium containing 5 μg/ml Alexa594-conjugated anti-mouse IgG antibody (Invitrogen) for 15 min. In these treatments, we recycled culture medium that neurons were grown in rather than using fresh culture medium. After a brief wash with external bath solution (see below), coverslips were immediately subjected to FM1-43 experiment. CFP was expressed by transfecting neurons with pCFP-N1 (Clontech) by calcium phosphate method at 7–10 DIV. Immunostaining for PSD-95 after live staining for GluR2 was carried out as follows. Neurons were fixed with 4% paraformaldehyde in PBS for 10 min and permeabilized with 0.1% Triton X-100, followed by blocking with 2% BSA and 1% normal goat serum. Neurons were then treated with 10 μg/ml anti-PSD-95 rabbit polyclonal antibody (Abcam) for 1 h and with 5 μg/ml Alexa488-conjugated anti-rabbit IgG antibody (Invitrogen).

FM1-43 Experiment. Coverslips were placed in a field stimulation chamber in which a pair of platinum wire electrodes was positioned 1 cm apart. AP was evoked by a 2 ms, 20 V square pulse. External bath solution contained (in mM) 137 NaCl, 5 KCl, 10 D-glucose, 5 Hepes (pH 7.3), 0.1 picrotoxin, 2 $CaCl_2$, 2 $MgCl_2$, 0.05 APV, and 0.01 CNQX, at 300 mOsm. $CaCl_2$ concentration was varied in some experiments. Neurons were loaded with FM1-43 (10 μM, Invitrogen) by 7 or 30 APs; 30 s after the end of stimulation, excess dye was washed with EBS containing 1 mM Advasep-7 (Biotium) for 1 min, then with EBS with 0.1 mM Ca^{2+} for 5 to 10 min. After replacing with normal EBS (2 mM Ca^{2+}), images were acquired before and after unloading stimulation by 600 APs at 20 Hz for three rounds with 15-s intervals. The remaining signals were taken as backgrounds and subtracted for the image analysis. Images were captured with a cooled CCD camera (CoolSNAP fx, Photometrics) attached to a BX50WI microscope (Olympus) with a $\times$60 water immersion lens (NA = 0.9, Olympus), a 470/50 nm excitation filter, a 500LP dichroic mirror, and a 545/75 emission filter and Metamorph software (Universal imaging corporation). ROI size was set to one. Neutral density filters were engaged to reduce the excitation light intensity to 25%.

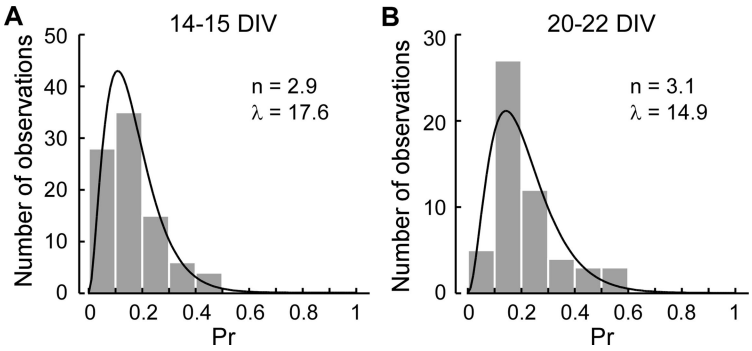

Fig. S1. Histogram of release probabilities. Shown are histograms of p_r estimated by the optical quantal analysis with FM1-43 in control conditions at 14–15 DIV (*A*, $n = 88$ boutons, 5 coverslips) and 20–22 DIV (*B*, $n = 54$ boutons, 5 coverslips). Histograms are fitted with gamma distribution curves of indicated parameters.

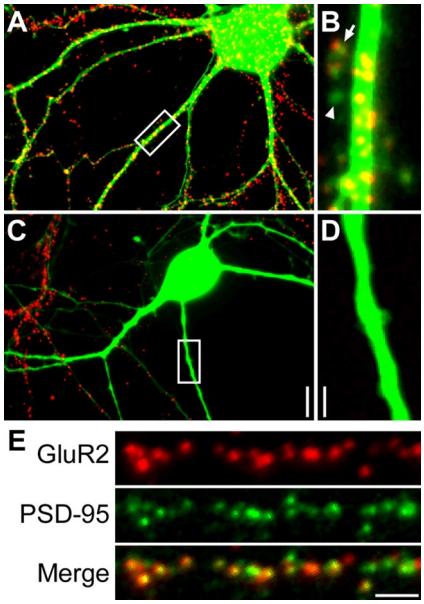

Fig. S2. GluR2 is expressed predominantly in spiny neurons, and is primarily synaptic. Cultured neurons were transfected with CFP and then surface GluR2 was live-labeled with anti-GluR2 extracellular domain antibody and Alexa594-conjugated anti-mouse IgG antibody. (*A*) Example image of a CFP-positive, GluR2-expressing neuron. CFP and GluR2 signals are shown in green and red, respectively. (*B*) Magnification of boxed region in *A*, showing this neuron is spiny. Note that some spines label for GluR2 (arrow), whereas other spines do not bear the GluR2 signal (arrowhead). (*C*) Example image of a CFP positive, GluR2-negative neuron. (*D*) Magnification of boxed region in *C*, showing the apparent lack of spines on this neuron. (*E*) Neurons live labeled for GluR2 at 12 DIV were fixed and double labeled for PSD-95. Majority of GluR2 puncta colocalized with PSD-95 puncta, showing synaptic origin of GluR2 signals. [Scale bars, 10 μm (*A* and *C*) and 2 μm (*B*, *D*, and *E*)].

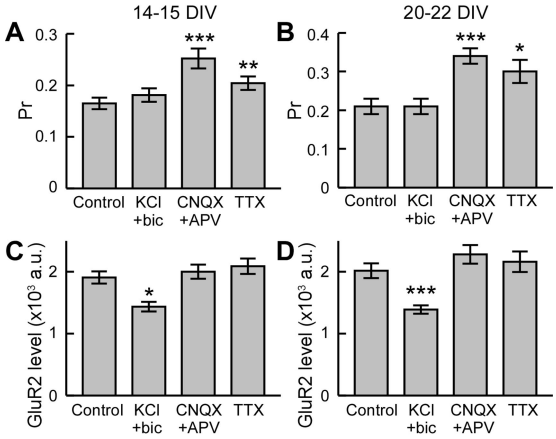

Fig. S3. Summary of the effect of TTX, CNQX, and APV, and KCl and bicuculline on p_r and GluR2 level. (*A* and *B*) Release probability in neurons treated for two days with KCl and bicuculline, TTX, or CNQX and APV at 14–15 DIV (*A*) and 20–22 DIV (*B*). (*C* and *D*) GluR2 level in neurons treated for two days with KCl and bicuculline, TTX, or CNQX and APV at 14–15 DIV (*C*) and 20–22 DIV (*D*). Data are shown as mean ± SEM. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, Steel's nonparametric multiple comparison test.

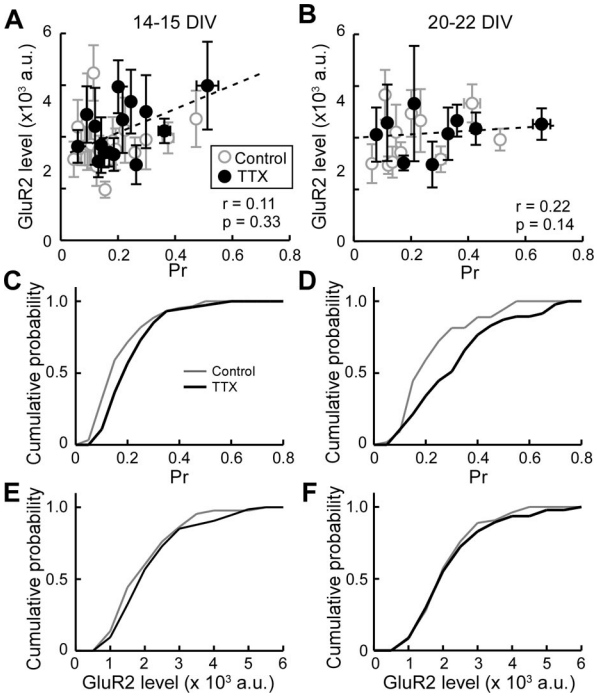

Fig. S4. Effect of activity blockade by TTX on the correlation between presynaptic release probability and postsynaptic GluR2 level. (*A* and *B*) Summary plots of GluR2 level versus release probability from 14–15 DIV neurons (*A*, $n = 74$ boutons, 6 coverslips) and 20–22 DIV neurons (*B*, $n = 48$ boutons, 4 coverslips) treated with 1 μM TTX for two days (black circles). Each point indicates an average from five to six boutons, grouped in the order of p_r. Dotted lines indicate linear regression fits. Data of the control experiment is also plotted (gray circles). Correlation coefficient (r) and p value (p) by Spearman rank correlation test are shown. TTX treatment does not significantly change the correlation. (*C* and *D*) Cumulative distribution plots of p_r at 14–15 DIV (*C*) and 20–22 DIV (*D*). Treatment with TTX increased p_r. K-S test, $P = 0.0027$ (14–15 DIV), $P = 0.0191$ (20–22 DIV). (*E* and *F*) Cumulative plots of GluR2 level at 14–15 DIV (*E*) and 20–22 DIV (*F*).