Supplementary Figure 1. Changes in paired pulse ratio (PPR) induced by perfusion with EphB2-Fc and ephrin-B1-Fc. (a) PPR was determined before and 30 min after the perfusion with control solution, CNQX (20 μM), APV (50 μM), showing no significant change. Note that the PPR was reduced by the perfusion with EphB2-Fc even in the presence of APV (APV + EphB2-Fc). (b) Time course of PPR changes after perfusion with the control solution, EphB2-Fc, and ephrin-B1-Fc. Sample traces of EPSCs are shown above. Scales: 30 pA, 15 ms. Significant difference is marked by “*” (*P < 0.05, Mann-Whitney U-test). Error bars represent s.e.m.
Supplementary Methods

**CV analysis of quantal release.** To determine the locus of the potentiation and depression induced by perfusion with the fusion protein, we measure the coefficient of variation ($CV$) of the EPSC amplitude, defined as $CV = \sigma / M$, where $\sigma$ and $M$ are the variance and the mean of the EPSC amplitude\(^1\). Assuming that synaptic transmission follow binomial statistics, the CV is related to the probability of release ($p$) for each of $N$ available quanta and the quantal size ($q$) by the formula: $(CV)^2 = Np / (1 - p)$, a quantity independent of $q$, hence postsynaptic changes in the responsiveness to glutamate. If synaptic modification involves only postsynaptic changes, $(CV)^2$ should not change after perfusion with the fusion protein. A plot of the ratio of $(CV)^2$ after and before perfusion should lie along the horizontal line equal to identity at all levels of synaptic changes (horizontal dotted line in **Fig. 5e**). Deviation of the data points away from the horizontal line suggests involvement of presynaptic mechanisms associated with changes in $p$, $N$ or both, depending on their positions relative to the diagonal line. Note that this interpretation does not take into account more complicated mechanisms, e.g., switching of postsynaptic sites between “on” and “off” states, due to gross insertion or removal of receptor clusters.

**Immunoprecipitation and Western Blotting.** To quantify the level of phosphorylated ephrin-B1 or EphB2 in the tadpole brain, we used protein precipitation procedures in conjugation with Western blotting to detect protein levels with ECL chemiluminescent (GE healthcare) detection procedure. Fusion proteins (0.5-2.0 nl of 200 ng/μl) were pressure-injected into the ventricle overlying the optic tectum. Two hours after injection, tadpole brains were dissected out manually under the microscope, and were lysed in ice-cold (4°C) lysis buffer (50 mM Tris-HCl, 150 mM
NaCl, 1% Nonidet P40, 0.5% sodium deoxycholate, pH7.5) with protease inhibitor cocktail (Roche Applied Science, Mannheim, Germany). Approximately 100 tadpole brains were collected to obtain 1 mg of total protein. The protein concentration of the lysates was measured using Bradford method (Bio-rad, Hercules, CA) and the total protein contents among various samples were equalized. Ephrin-B1 or EphB2 was immunoprecipitated from 2 mg total protein/ml brain lysate using a goat antibody directed against the extracellular domain of ephrin-B1 or EphB2 (R & D systems, Inc.). After overnight incubation and subsequent precipitation of the ephrin-B1- or EphB2-antibody complex with Protein G-Agarose (Roche Applied Science, Mannheim, Germany), the precipitated protein complexes were washed in three changes of cold lysis buffer containing 500 mM NaCl. Ephrin-B1 or EphB2 and antibody were dissociated by heating for 5 min in Laemmli sample buffer (Bio-rad, Hercules, CA) before loading onto 10% SDS polyacrylamide gels. After transfer to nitrocellulose filters, the filters were blocked with 5% skim milk in 0.1 M PBS and then incubated overnight at 4°C with the appropriate primary antibody. The filters were developed using ECL chemiluminescence reagents (GE healthcare) with secondary antibodies tagged with HRP (Horse radish peroxidase).

**Electron Microscopy.** Stage 45 tadpoles were anesthetized and fixed in 3.5% paraformaldehyde, 0.2 % glutaraldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The brains was dissected and fixed for 1 h at 4°C, and the optic tectum was then sectioned coronally at 50 µm. Sections were treated with PB containing 0.05% Triton X-100 to improve reagents penetration, and blocked in 0.1 M PBS (pH 7.5) containing 0.5% acetylated bovine serum albumin (BSA-c, Aurion, Wageningen, Netherlands), and then incubated overnight in a primary rabbit polyclonal antibody against ephrin-B1 or EphB2 (purified by IgG purification kit, Pierce Biotech.; 1:10 dilution in 0.2%
BSA-c in PBS, Santa Cruz Biotech.), followed by 3-h incubation in a secondary goat anti-rabbit IgG coupled to 1 nm gold particles (1:50 dilution in 0.2% BSA-c in PBS; pH 7.4). Further fixation of the sections was done with incubation in 2.5% glutaraldehyde in PB for 2 h and gold particles were enlarged using a R-GENT SE-EM Silver Enhancement Reagent (Aurion). Sections were then washed with PB, fixed with 1% osmium tetroxide for 2 h, dehydrated, and flat-embedded in Eponate 12 resin (Ted Pella; Redding, CA) between two sheets of Aclar film (EM Sciences; Fort Washington, PA). After resin polymerization, 60 nm sections were obtained using an ultra-microtome with a diamond knife (Diatome), counterstained with 2% uranyl acetate and lead citrate, and viewed by a transmission electron microscope (Tecnai 12, FEI Company, Hillsboro, OR).

Reference