**Supporting Information**

**Heine et al. 10.1073/pnas.0804007106**

**SI Methods**

**Molecular Constructs and Reagents.** To make PSD-95-GFP and mCherry constructs, the mCherry gene obtained from R. Tsien (University of California, San Diego, CA) was amplified by PCR with primers containing AgeI/NheI sites. It was then inserted at position +253 aa in the PSD-95 pcDNA from S. Okabe (Tokyo University, Tokyo, Japan) (1), which was also modified to contain AgeI/NheI sites at the respective positions. To make the PSD-95-GFP construct, mCherry was replaced at these positions by the GFP sequence. Homer1c-GFP was also a gift from S. Okabe. GluR1-GFP and GluR2-GFP constructs were described previously (2).

The neuriligin constructs Nlg1 and Nlg1-SWAP in which the extracellular domain was replaced by acetycholine esterase were gifts from P. Scheiffele (Columbia University, New York, NY) (3). To make Nlg1-mCherry, Nlg1 was sub-cloned into pcDNA between HindIII and NotI sites, and the mCherry cassette was inserted at position -20 aa from the stop codon, following the same strategy as for PSD-95-mCherry.

The pcDNA neomycin βNeurexin1Δ4-humanFc (Nrx1β-Fc) plasmid, also received from P. Scheiffele (4), was sub-cloned between sites HindIII/XhoI in pcDNAhygro (+) vector to select a stable hygromycin-resistant HEK cell line producing Nrx1β-Fc. Recombinant Nrx1β-Fc protein was expressed in HEK cells and purified from conditioned medium as described (5). N-cadherin fused to human Fc (Ncad-Fc) described earlier (6) was a gift of M. Lambert and R.M. Mége (INSERM, Paris, France). Globulin-free BSA and other chemicals were purchased from Sigma. Pharmacological compounds (CNOX, APV, TTX, and bicuculline) were from Tocris.

**Cell Culture and Transfection.** Dissociated hippocampal neurons from E18 rat embryos were plated on 18-mm polylysine-coated glass coverslips at a density of 10’000 cells/cm² in MEM containing 10% horse serum (Gibco) for 3 h, then cultured in Neurobasal medium supplemented with B27 on a layer of glial cells (7). Neurons were transfected at 4 or 5 DIV using Effecten (Qiagen) and processed 2 to 3 days later.

**Microsphere Coating.** Fifty microliters of 4-μm tosyl paramagnetic microspheres (Dynal) or 10 μL of 2-μm latex (Interfacial Dynamics) were incubated overnight at 4 °C with 20 μg goat anti-human Fc antibody (Jackson Immunoresearch), followed by 3 h incubation with 5 μg purified Nrx1β-Fc, N-cadherin-Fc, or human Fc (Jackson Immunoresearch). This procedure led to an approximate coating density of 500 molecules/μm² (8). Each coverslip carrying neurons was incubated with 5 μL of bead solution mixed to 1 mL culture medium containing 1% BSA for varying time durations.

**Immunocytochemistry.** To quantify PSD-95 recruitment, cells co-transfected with Nlg1 and PSD-95-GFP and carrying Nrx1β-Fc or N-cadherin-Fc beads were fixed for 10 min in warm 4% paraformaldehyde/4% sucrose in PBS solution, and remaining active sites were saturated with 50 mM NH₄Cl in PBS solution for 15 min. Cells were permeabilized with 0.3% Triton X-100 in PBS solution for 5 min, and non-specific binding sites were blocked with 1% BSA in PBS solution for 30 min. For the time course of PSD-95 accumulation, cells were incubated in PBS-BSA with 1:1,000 rabbit anti-GFP (Invitrogen) for 1 h, rinsed and incubated with 1:1,000 Alexa 488-conjugated goat anti-rabbit antibody (2 mg/mL; Molecular Probes) for 30 min, and mounted in Vectashield (Vector Laboratories). For visualization of presynapses, neurons co-transfected with Nlg1 and PSD-95-mCherry were treated as described with 1:400 primary rabbit anti-synaptotagmin antibody (a gift of C. Dotti, Leuven University, Leuven, Belgium). For assessment of endogenous AMPAR recruitment, neurons co-transfected with Nlg1 and PSD-95-mCherry were incubated at 37 °C for 20 min with 1:100 polyclonal rabbit anti-GluR1 antibody (a gift of R. Huganir, Johns Hopkins University, Baltimore, MD), then rinsed, fixed, and stained without permeabilization with 1:500 Alexa 488-conjugated goat anti-rabbit antibody. For the live staining of recombinant AMPAR subunits GluR1-GFP and GluR2-GFP, neurons were incubated with 1:400 monoclonal anti-GFP (Roche) for 10 min at 37 °C, then fixed, permeabilized, and further stained with 1:400 guinea pig anti-PSD-95 antibody (a gift of M. Sheng, MIT, Boston, MA) followed by a mixture of 1:1,000 Alexa 488-conjugated anti-mouse and 1:1,000 Alexa 568-conjugated anti-guinea pig antibodies (Invitrogen).

**Image Analysis.** Immunostainings were visualized on an epifluorescence microscope (IX 70; Olympus) equipped with a ×100/1.4 NA objective and appropriate filter sets (Chroma). Images were acquired with a CCD camera (HQ CoolSnap; Roper Scientific). Using the software Metamorph (Universal Imaging), we quantified an enrichment factor, defined as the ratio of fluorescence level at the bead surface versus that on a nearby control region on the same neurite, for an average of three beads per cell, showing the highest accumulation. To quantify the enrichment of recombinant AMPAR subunits at endogenous synapses, the PSD-mCherry images were treated by a segmentation program written within Metamorph (9), allowing precise detection of clusters. The corresponding contours were transferred to the GFP image, and the GluR1 or GluR2 intensity was measured in these regions and normalized by that on a control area.

**Glutamate Un-Caging.** Cells transfected with PSD-95-mCherry and carrying Nrx1β-Fc latex beads were loaded with 5 μM Fluo-4-AM (Molecular Probes) for 15 min in culture medium, then mounted in HBS containing 2 mM calcium and 0 mM magnesium (10) and supplemented with 3 mM 4-methoxy-7-nitroindolinyl-caged l-glutamate (Tocris), 10 μM glycine as a co-agonist of NMDARs, and 1 μM TTX to prevent action potentials. In control experiments, 4-methoxy-7-nitroindolinyl-caged l-glutamate was omitted. The observation chamber was placed on a confocal scanning laser microscope (TCS SP2; Leica) equipped with a pulsed bi-photon laser (Mira 900; Coherent) tuned to 750 nm and set to 100 mW by an electro-optic modulator (Linos Photonics). The optimal laser power was adjusted on a control sample made of DMNP fluorescein (Sigma) trapped in micro-islets formed in polymerized Sylgard (11), so as to yield rapid and reproducible increases in fluorescence with minimal photo-damage. A zoomed area of 6 × 6 μm around a bead was scanned at 800 Hz by the 488-nm Argon laser line and the fluorescence between 500 and 530 nm was collected by a photomultiplier, using a ×60/1.3 objective and a pinhole open to three times the Airy disk (180 μm), at a frequency of 5 Hz. During a 20-sec fluorescence recording, one pulse of bi-photon light corresponding to one scan duration (200 ms) was given using a shutter (Uniblitz). The PSD-mCherry fluorescence (laser excitation, 542 nm; emission, 560–650 nm) and transmitted light intensity were also recorded. Conveniently, the bi-
photon pulse caused sudden photo-bleaching of mCherry and appeared as a stimulation artifact in the transmission image. For pharmacological treatments, 100 μM D-AP5 (Advent), 20 μM CNQX (Tocris), or 200 μM cadmium chloride (Sigma) were added to the extracellular mounting solution.

**Electrophysiology.** The extracellular solution contained (in millimolar concentrations): 145 NaCl, 2.5 KCl, 0 MgCl₂, 2 CaCl₂, 10 Hepes, and 10 D-Glucose, pH 7.4, supplemented with 10 μM bicuculline (Tocris) to block GABA-A receptors and 1 μM TTX. When indicated, 10 μM CNQX or 10 μM APV were added. Borosilicate cylinders (Clark Electromedical) were pulled with a micropipette puller (Sutter Instruments) to produce patch electrodes with resistances of 3 to 5 MΩ. The intracellular solution contained (in millimolar concentrations): 130 CH₃C₅SO₃, 2 MgCl₂, 1 CaCl₂, 4 NaATP, 10 EGTA, 10 Hepes, and 0.4 GTP, pH 7.25. Micropipette positioning was achieved through three-axis micromanipulators (Sutter Instruments) under an inverted epifluorescence microscope equipped with a x60/1.35 objective (IX70; Olympus). Recordings were performed with an EPC 10 double patch-clamp amplifier (HEKA Electronics). Data were acquired and stored using Pulse-Pulse fit software (version 8.62; HEKA Electronics) and analyzed with Igor (Wavemetrics) and GraphPad Prism software. Local activation of receptors was performed by glutamate iontophoresis (2), using pipettes with a resistance of 40 to 60 MΩ filled with 150 mM sodium glutamate, pH 7.4, and connected to an amplifier from NPI Electronics. A small retaining current was needed to keep glutamate inside the pipette (usually 10–50 nA). Current pulses of 200 to 300 nA and 1 ms duration were applied to evoke glutamate receptor-mediated currents. At least 10 recorded current traces were averaged. The averaged current curves were fitted by bi-exponential functions using the Igor software (Wavemetrics), from which the relative charge carried by the fast AMPAR component was computed.

Fig. S1. TTX/APV treatment increases AMPAR recruitment at synapses. Neurons co-transfected with Nlg1 and PSD-95-mCherry were incubated without (a) or with (b) TTX/APV for 24 h and processed for GluR1 surface staining. (c) The enrichment level of GluR1 at PSD-mCherry-positive clusters (arrowheads) was quantified as described in Materials and Methods.
Fig. S2. Electrophysiological properties of the currents recorded at Nrx1β-Fc beads and synapses. (a) Average current/time curve for 2–4 h and 24 h Nrx1β-Fc bead contacts and endogenous PSD-95-GFP clusters in cells transfected with Nlg1 and PSD-95-GFP. (b) Rectification index calculated as the slope of the current/voltage curves.
Fig. S3. Surface immunolabeling of endogenous AMPARs at bead contacts. Neurons co-transfected with Nlg1 and PSD-95-mCherry were incubated for 24 h with either Nrx1β-Fc (a) or N-cadherin-Fc (b)-coated beads, then processed for live AMPAR immunostaining by using an antibody against the N-terminal domain of GluR1. GluR1 co-distributed with PSD-95 at Nrx1β-Fc beads, whereas neither PSD-mCherry nor GluR1-containing AMPAR accumulated at Ncad-Fc beads. (c and d) Quantification of the enrichment levels of PSD-95-mCherry and GluR1, respectively, for both types of beads.