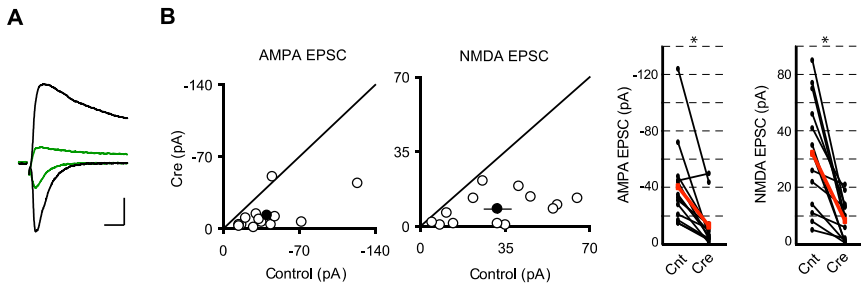
Supplementary Material:

The cell-autonomous role of excitatory synaptic transmission in the regulation of neuronal structure and function

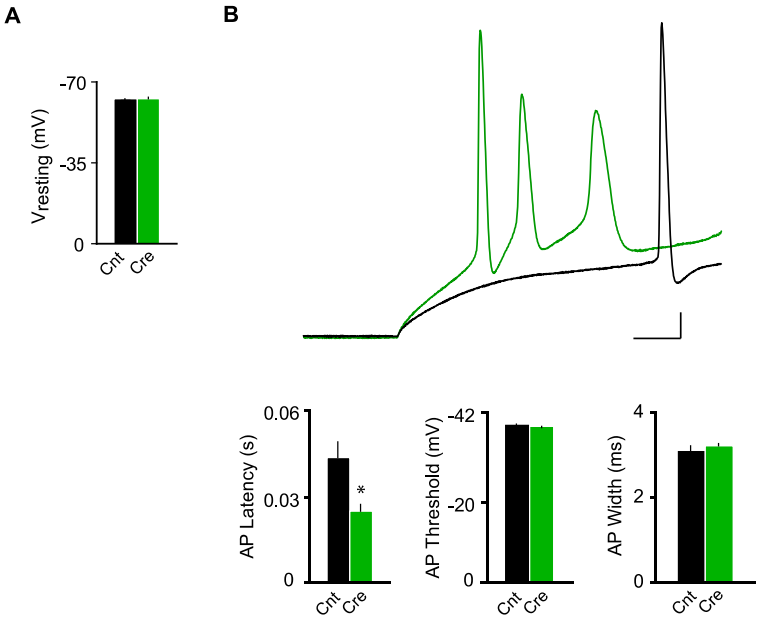
Wei Lu, Eric A. Bushong, Tiffany P. Shih, Mark H. Ellisman and Roger A. Nicoll

Supplemental Figures:

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure Legends:

Supplemental Figure 1. Dramatic reduction of AMPAR- and NMDAR- mediated synaptic transmission onto CA1 pyramidal neurons from *GRIA1-3*$^{fl/fl}$*GRIN1*$^{fl/fl}$ mice expressing CreGFP for 10 days *in vivo*.

(**A**) Representative sample traces of dual whole-cell voltage-clamp recording in acute hippocampal slices from p11-p12 *GRIA1-3*$^{fl/fl}$*GRIN1*$^{fl/fl}$ mice infected with virus expressing CreGFP at p0. (Black, Control (Cnt); Green, Cre). Scale bar: 0.02 s, 50 pA. (**B**) Scatter plots showed amplitudes of EPSCs for single pairs (open circles) and mean ± SEM (filled circles) (Cnt: -40.62 ± 8.25 pA; Cre: -12.92 ± 4.34 pA; n = 13; **P* < 0.002) and NMDA EPSCS (Cnt: 32.08 ± 5.51 pA; Cre: 8.38 ± 2.01 pA; n = 13; **P* < 0.001). Graphs in the right show that synaptic transmission mediated by AMPARs or NMDARs is significantly reduced in CreGFP-expressing neurons (each black line represents a single pair and the red line represents the average).

Supplemental Figure 2. Characterization of action potential in CA1 pyramidal neurons from *GRIA1-3*$^{fl/fl}$*GRIN1*$^{fl/fl}$ mice expressing CreGFP.

(**A**) Resting membrane potentials from Cnt or Cre-expressing CA1 pyramidal neurons (Cnt: -62.64 ± 0.59 mV, n = 11; Cre: -62.75 ± 0.64 mV, n = 12; *P* = 0.9). (**B**) Overlapped traces recorded from Cnt or Cre-expressing CA1 pyramidal neurons show Cre-expressing neurons (green trace) have similar voltage threshold for action potential (Cnt: -39.29 ±

0.89 mV, n = 7; Cre: -38.73 ± 1.2 mV, n = 11; *P* = 0.74) and action potential width (Cnt: 3.13 ± 0.07 ms, n = 8; Cre: 3.19 ± 0.06 ms, n = 13; *P* = 0.55), but have an earlier onset (Cnt: 43.38 ± 6 ms, n = 8; Cre: 24.83 ± 2.75, n = 12; **P* = 0.006) for the 1st action potential on step-current injection compared with control neurons (black trace). Error bars represent the mean ± SEM.

Materials and Methods:

Mice Genetics

Animal housing were performed according to the university guidelines at the University of California at San Francisco. Quadruple conditional mice *GRIA1-3*$^{fl/fl}$*GRIN1*$^{fl/fl}$ were generated by crossing triple *GRIA 1-3*$^{fl/fl}$ mice with *GRIN1*$^{fl/fl}$ mice as described before (Lu et al., 2009; Lu et al., 2011).

Electrophysiology

GRIA1-3$^{fl/fl}$*GRIN1*$^{fl/fl}$ mice were injected at 0-1d after birth (P0-P1) with high-titer rAAV-GFP-Cre viral stock (~1-5x10e12 vg/ml) as described before (Lu et al., 2009). Three to four weeks after injection, transverse 300μm hippocampal slices were dissected out from mice and cut on a Leica vibratome in high sucrose cutting solution, containing (in mM): NaCl 50, KCl 2.5, $CaCl_2$ 0.5, $MgCl_2$ 7, NaH_2PO_4 1.0, $NaHCO_3$ 25, glucose 10 and sucrose 150. Freshly cut slices were placed in an incubating chamber containing artificial cerebrospinal fluid (ACSF), containing (in mM) NaCl 119, KCl 2.5, $NaHCO_3$ 26, Na_2PO_4 1, glucose 11, $CaCl_2$ 4, $MgCl_2$ 4, and recovered at 35 °C for ~1h. Slices were then maintained in ACSF at room temperature prior to recording. After 0.5-1 h of incubation at room temperature slices were transferred to a submersion chamber on an upright Olympus microscope, perfused in normal ACSF saturated with 95% O_2/5% CO_2. For recording AMPA and NMDA EPSCs, picrotoxin (0.1mM) and bicuculline (0.01mM) were added to ACSF; for recording GABA EPSCs, APV (0.1mM) and NBQX (0.02mM) were added to ACSF. The intracellular solution for voltage clamping recording contained

(in mM) $CsMeSO_4$ 135, NaCl 8, HEPEs 10, Na_3GTP 0.3, MgATP 4, EGTA 0.3, QX-314 5, and spermine 0.1. Cells were recorded with 3- to 5-MΩ borosilicate glass pipettes, following stimulation of Schaffer collaterals with monopolar glass electrodes filled with ACSF placed in stratum radiatum at the CA1 region. For current clamping recording, the intracellular solution contained (in mM) KMeSO4 130, KCl 10, HEPES 10, NaCl 4, EGTA 1, Mg-ATP 4, and Na-GTP 0.3. The cell-attached recording in Figure 2C was performed in ACSF containing $CaCl_2$ 2.5mM and $MgCl_2$ 1.3mM. Resting membrane potentials were recorded under I = 0 configuration. Input resistance was determined with measuring current/voltage relationships through injecting negative currents into the cells with potassium based intracellular solution. Action potentials were generated by injection of step-depolarizing current pulses. Series resistance was monitored and not compensated, and cells in which series resistance varied by 25% during a recording session were discarded. Synaptic responses were collected with a Multiclamp 700A-amplifier (Axon Instruments, Foster City, CA), filtered at 2 kHz, digitized at 10 Hz.

GFP positive neurons were identified by epifluorescence microscopy. All paired recordings involved simultaneous whole-cell recordings from one GFP positive neuron and a neighboring GFP negative neuron. The stimulus was adjusted to evoke a measurable, monosynaptic EPSC in both cells. AMPAR EPSCs were measured at a holding potential of − 70 mV, and NMDAR EPSCs were measured at +40 mV and at 100 ms after the stimulus, at which point the AMPAR EPSC has completely decayed. $GABA_AR$ IPSCs were measured at +40 mV in Figure 2A. AMPA mEPSCs and GABA mIPSCs were acquired in the presence of 0.5-1 μM TTX, and semiautomatically detected

by offline analysis using in-house software in Igor Pro (Wavemetrics). All paired recording data were analyzed statistically with a two-tailed paired Student t test. For all other analyses an unpaired 2-tailed t-test was used. All errors bars represent standard error measurement.

Anatomy and Imaging

For spine analysis, a 3-4 week old mouse injected with rAAV-GFP-Cre at p0 was anesthetized with Nembutal and then perfused with Ringer's solution, followed by 4% paraformaldehyde (PFA) in PBS, subsequently followed by a brief post-fixation of brain on ice. 100 μm thick slices were cut on vibrating microtome. GFP (+) and (-) cells were identified by epifluorescence and were iontophoretically filled with Alexa Fluor 568 using a sharp micropipette. Slices were post-fixed in 4% PFA and then cover-slipped. Confocal stacks of pyramidal neuron dendrites filled with Alexa 568 were collected on an Olympus FV1000 confocal microscope using a 60x oil objective (NA 1.42) and a step size of 0.4 um. For dendrite morphology analysis, CA1 pyramidal cells in acute slices were filled with Alexa Fluor 568 dyes through the patch pipette for about 10 min. After filling, slices were fixed in 4% PFA/4% sucrose in PBS for 30 min at room temperature, followed by washing at least three times with PBS. Neurons were imaged with 25X objective on a Zeiss confocal microscope (NA 0.8, LSM5 Pascal, Germany). Spine density and apical dendritic length were measured using Neurolucida (MBF Bioscience, Williston, VT). Spine density was measured along 15-30 um segments of 3-4 secondary/tertiary dendrites and averaged for each neuron. The spine lengths and widths were measured in Neurolucida. Each dendrite was traced to accurately reflect dendrite

diameter and the length and width of each spine was manually measured for each spine. For statistical analysis Mann Whitney U test was used, and data were presented as mean ± SEM.

Electron Microscopy

Six mice were anesthetized with Nembutal and then transcardially perfused with 4% PFA and 0.05% glutaraldehyde in PBS. Cells were filled with 5% Lucifer Yellow as described above. Slices were post-fixed with 4% PFA / 0.05% glutaraldehyde for 30 minutes. The slices were placed in 2% glutaraldehyde in PBS for 5 minutes. The slices were rinsed with PBS and placed into 100 mM glycine-PBS for 5 minutes. The slices were rinsed and placed into PBS containing 0.15% diaminobenzidine and 0.1% potassium cyanide. The slice was illuminated using an epifluorescence adapter on a Zeiss Axiovert using a 63x oil objective (NA 1.25) and a xenon lamp using a Lucifer Yellow filter cube (425/40, 460DC, 540/50) (Chroma, Bellows Falls, VT) until the dye-filled cell turned light brown. The slices were washed in PBS on ice and then fixed in 1% OsO4 in PBS on ice for 45 min. The slices were washed in water and then placed in 2% aq. uranyl acetate in fridge overnight. The slices were washed with water and then dehydrated with a series of ethanol (70%, 90%, 2x 100%) and acetone (2x 100%) solutions for 10 minutes each step. The slices were infiltrated with Durcupan ACM resin (Sigma, St Louis, MO) and polymerized at 60 degrees for 48 hours.

Blocks containing photoconverted neurons were trimmed and serial ultrathin sections (70 nm) were collected on formvar-coated slot grids. The sections were stained with 1% aq.

uranyl acetate and Sato lead and then imaged on an FEI Spirit at 100 kV and magnification of 11,000X on a 2k x 2k CCD camera (TVIPS, Gauting, Germany). Image analysis was performed in IMOD (Kremer et al., 1996). The RRP was quantified by measuring all vesicles through serial reconstruction of spines located within 100 nm of pre-synaptic membrane opposite PSD.

Reference

1 Lu, W., Shi, Y., Jackson, A.C., Bjorgan, K., During, M.J., Sprengel, R., Seeburg, P.H., and Nicoll, R.A, *Neuron* **62**, 254 (Apr 30, 2009).

2 W. Lu, J. A. Gray, A. J. Granger, M. J. During, R. A. Nicoll, *J Neurophysiol* **105**, 923 (Feb, 2011).