

# Supporting Information

Zhang et al. 10.1073/pnas.0802940105

## SI Text

**Plasmid Construction.** Chr2-YFP, a gift from K. Deisseroth, was modified by inserting a stop codon (TAG) after amino acid 309 by PCR and inserted into a synapsin-I (syn) promoter vector (1). The fragment of syn-ChR2-SV40-polyA was excised and inserted into a second expression vector containing syn-RFP (tdimer2, a dimeric red fluorescent protein from R.Y. Tsien), resulting in a single plasmid for neuron-specific coexpression of 2 proteins. GFP- $\alpha$ CaMKII (from T. Meyer) was subcloned into a separate syn promoter vector, and new  $\alpha$ CaMKII fusions were made by replacing GFP with YFP or Dronpa-Green (MBL, Naka-ku Nagoya, Japan). Mutant  $\alpha$ CaMKII (TTVA) was generated by site-directed mutagenesis (Thr<sup>305</sup>  $\rightarrow$  Val and Thr<sup>306</sup>  $\rightarrow$  Ala) using the QuikChange kit (Stratagene). All constructs were verified by DNA sequencing, amplified, and purified using MaxiPrep kits (Qiagen).

**Slice Culture and Transfection.** Organotypic hippocampal slices were prepared from Wistar rats at postnatal day 5 as described in ref. 2, in accordance with the animal care and use guidelines of the Veterinary Department Basel-Stadt. After 7 days *in vitro*, cultures were transfected with syn-ChR2-syn-RFP in combination with syn- $\alpha$ CaMKII fused to GFP, YFP, or Dronpa-Green, using a Helios Gene Gun (BioRad). All experiments were performed 2–3 weeks after transfection. At this time, total  $\alpha$ CaMKII in transfected pyramidal cells was increased by only  $\approx$ 12% relative to neighboring untransfected cells (Fig. S2).

**Electrophysiology.** Hippocampal slice cultures were placed in the recording chamber of the microscope and superfused with artificial cerebrospinal fluid (ACSF) containing (in mM): 119 NaCl, 2.5 KCl, 4 CaCl<sub>2</sub>, 4 MgCl<sub>2</sub>, 26.2 NaHCO<sub>3</sub>, 1 NaH<sub>2</sub>PO<sub>4</sub>, and 11 glucose. The solution was gassed with 95% O<sub>2</sub>, 5% CO<sub>2</sub>, and pH was adjusted to 7.2. Single and dual whole-cell recordings were performed using Axopatch 200B and MultiClamp 700B amplifiers (Axon Instruments). Recording pipettes (4.5–5.5 M $\Omega$ ) were filled with intracellular solution containing (in mM): 135 K-gluconate, 10 Hepes, 4 MgCl<sub>2</sub>, 4 Na<sub>2</sub>ATP, 0.4 Na<sub>2</sub>GTP, 10 Na<sub>2</sub>-phosphocreatine, 3 ascorbate, and pH was adjusted to 7.2. LTP of unitary EPSCs was induced by repeatedly pairing (20 times, 0.1 Hz) presynaptic stimulation (2 nA, 5 ms) with a postsynaptic burst of 4–9 action potentials induced by blue light stimulation (200 ms). For uncaging experiments, MNI-caged-L-glutamate (Tocris) was bath applied at 5 mM in ACSF containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, 26.2 NaHCO<sub>3</sub>, 1 NaH<sub>2</sub>PO<sub>4</sub>, 11 glucose, 0.03 D-serine, and 0.005 2-chloroadenosine. LTP of uncaging-evoked EPSCs was induced by repeatedly pairing (20 times, 0.1 Hz) uncaging of glutamate with blue light stimulation (200 ms) of the postsynaptic cell. For tetanic stimulation experiments, bipolar electrodes (FHC, ME) were placed in stratum radiatum  $\approx$ 200  $\mu$ m lateral to the recording site, and 10  $\mu$ M bicuculline and 4  $\mu$ M 2-chloroadenosine were added to the ACSF to facilitate LTP induction and avoid recurrent excitation. All recordings were performed at 30–32°C.

**Light Stimulation, 2-Photon Imaging, 2-Photon Uncaging.** The 2-photon imaging and uncaging setup was based on a Olympus BX51WI microscope equipped with a LUMFL 60 $\times$  1.1 NA objective, controlled by a free software package (3) written in Matlab (The MathWorks). Two ultrafast IR lasers (Chameleon-XR, Coherent; Mai Tai HP, Spectra-Physics) controlled by electro-optic modulators (350–80, Conoptics) were combined by a polarizing beamsplitting cube (Thorlabs) for simultaneous imaging (930 nm) and uncaging (725 nm). A blue LED (470 nm, Cairn Research) was coupled into the epifluorescence pathway to deliver light pulses for ChR2 activation (Fig. 2a). Two PMTs (R3896, Hamamatsu) below a 1.4 NA oil-immersion condenser were used to detect red and green emission. During the blue light pulse, they were protected by a VS25 shutter (Vincent Associates). Glutamate uncaging was achieved using a 0.5 ms pulse of 725 nm light. Laser intensity was adjusted to mimic the amplitude of miniature EPSCs.

**Image Analysis.** Off-line analysis was performed using custom routines written in Matlab. We used the ratio of green/red fluorescence ( $G/R$ , or  $Y/R$  in case of YFP- $\alpha$ CaMKII) as a measure of  $\alpha$ CaMKII concentration. We verified that no pixel in either channel was saturated. To display ratio images, we used a hue/saturation/brightness color model, where hue was determined by the  $G/R$  ratio of every pixel (using a rainbow color table), and brightness was set by the intensity in the red (volume) channel. For quantitative analysis, we calculated the  $G/R$  ratio in a region of interest after subtraction of background fluorescence and optical cross-talk. To compensate for differences in the relative expression levels of the two constructs in different cells, we normalized the  $G/R$  ratio in the spine by the  $G/R$  ratio in the dendrite to get the spine/dendrite ( $S/D$ ) ratio.

$$S/D = \frac{G_{spine}/R_{spine}}{G_{dendrite}/R_{dendrite}} = \frac{[CaMKII]_{spine}}{[CaMKII]_{dendrite}}. \quad [1]$$

To calculate the bound fraction from the  $S/D$  ratio, we assumed that the concentration difference between spine and dendrite was due to bound CaMKII in the spine:

$$[CaMKII]_{spine,bound} = [CaMKII]_{spine} - [CaMKII]_{dendrite} \quad [2]$$

$$[CaMKII]_{spine,bound} = [CaMKII]_{spine} - \frac{[CaMKII]_{spine}}{S/D} \quad [3]$$

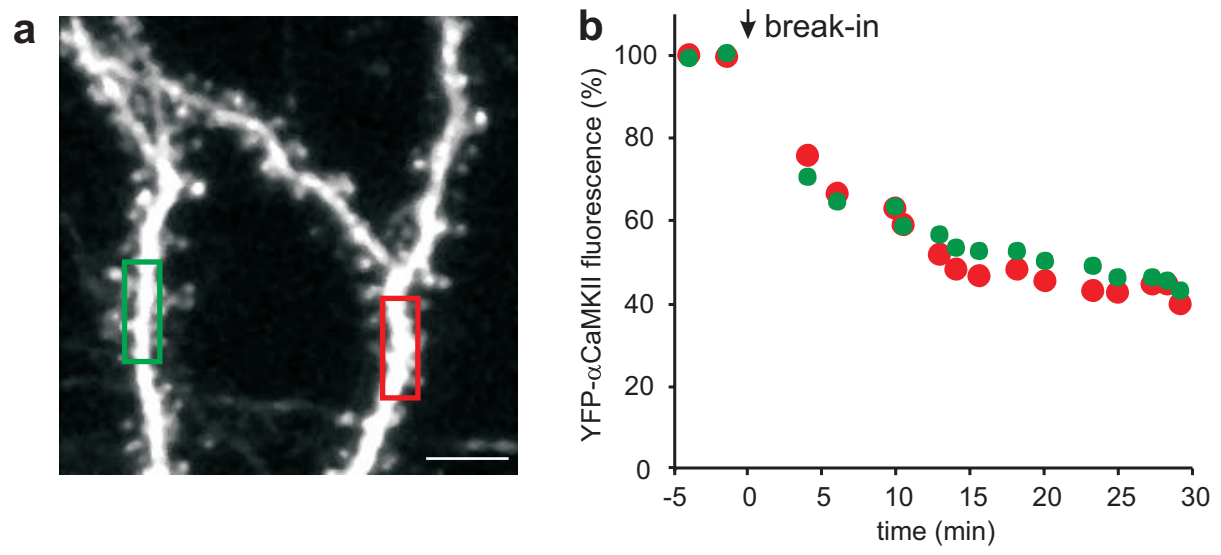
or, expressed as a fraction of total  $[CaMKII]_{spine}$

$$[CaMKII]_{spine,bound} = 1 - \frac{1}{S/D} \quad [4]$$

Average values are given as mean  $\pm$  SEM if not indicated otherwise. Significance was defined as  $P < 0.05$  and determined using Student's *t* test (two-tailed) if not indicated otherwise.

1. Kugler S, Meyn L, Holzmüller H, Gerhardt E, Isenmann S, et al. (2001) Neuron-specific expression of therapeutic proteins: Evaluation of different cellular promoters in recombinant adenoviral vectors. *Mol Cell Neurosci* 17:78–96.  
2. Stoppini L, Buchs PA, Müller D (1991) A simple method for organotypic cultures of nervous tissue. *J Neurosci Meth* 37:173–182.

3. Pologruto TA, Sabatini BL, Svoboda K (2003) ScanImage: Flexible software for operating laser scanning microscopes. *Biomed Eng Online* 2:13.

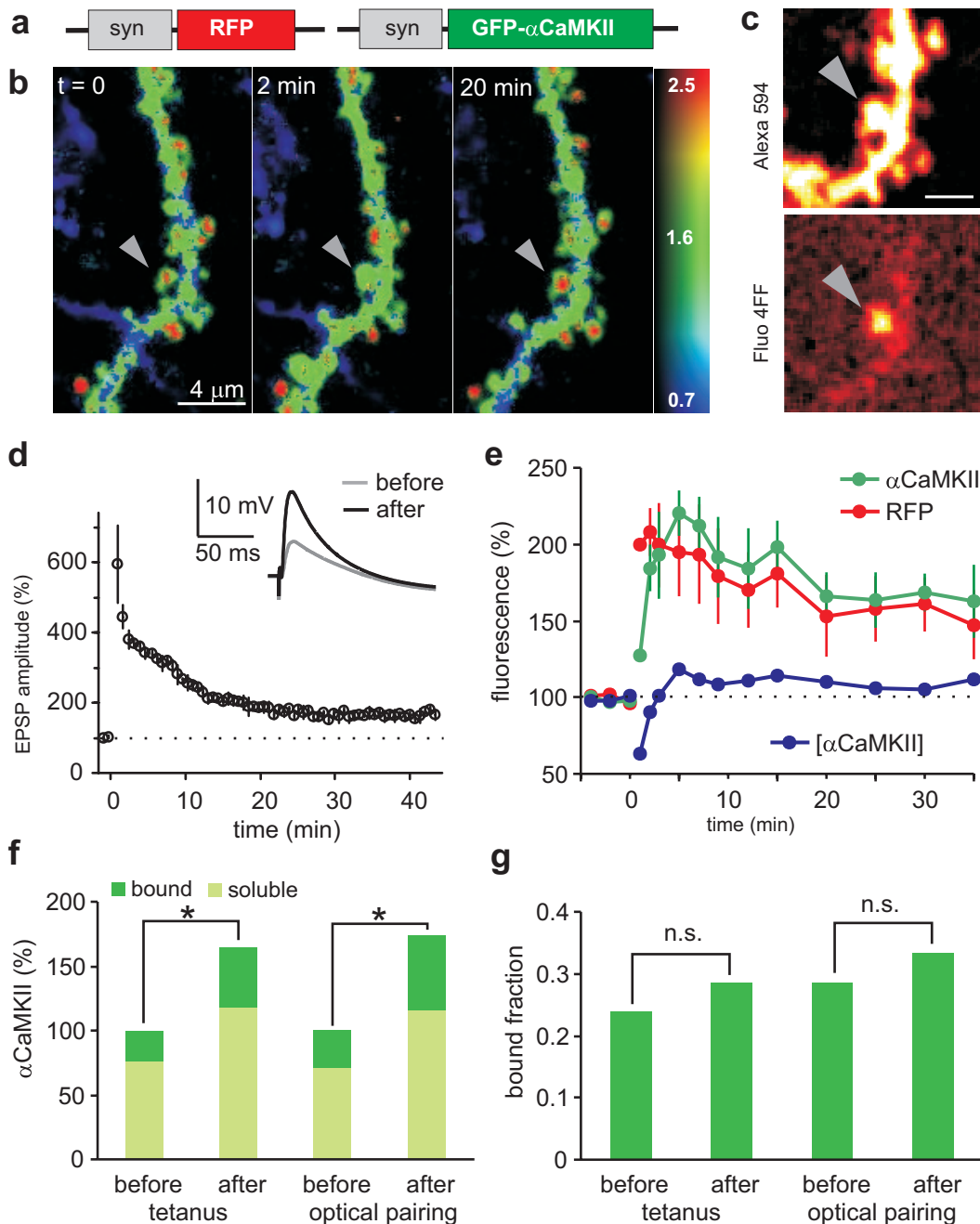


**Fig. S1.** Example of  $\alpha$ CaMKII wash-out after whole-cell patch clamp. (a) Image of YFP- $\alpha$ CaMKII transfected cell before patch clamp. CA1 pyramidal cell, oblique dendrites. Scale bar: 5  $\mu$ m. (b) Decay of YFP- $\alpha$ CaMKII fluorescence in oblique dendrites after formation of the whole-cell configuration at  $t = 0$ .









**Fig. 55.** Input-specific accumulation of  $\alpha$ CaMKII induced by high-frequency stimulation of Schaffer collaterals. (a) Schematic drawing of the two vectors used for double transfection by gene gun. Promoter denotes synapsin-I promoter. (b) Ratio images of cells expressing YFP- $\alpha$ CaMKII and RFP before ( $t = 0$ ) and after (2 min, 20 min) repetitive stimulation of Schaffer collateral fibers at high frequency ( $3 \times 100$  Hz, 1 s, at 0.1 Hz). Warm colors indicate high  $\alpha$ CaMKII concentration. At  $t = 0$ , the high frequency protocol was applied. The spine marked by the arrowhead was the stimulated spine identified by post hoc calcium imaging (see b). (c) Post hoc identification of the stimulated spine using calcium imaging. Upper image: Alexa Fluor 594 fluorescence. Lower image: Fluo 4FF signal ( $\Delta f$ ) after synaptic stimulation at depolarized membrane potential (0 mV), indicating localized calcium influx at stimulated spine. Scale bar:  $2 \mu\text{m}$ . (d) Tetanic stimulation induces LTP at Schaffer collateral synapses. Gray bar indicates high-frequency stimulation ( $3 \times 1$  s, 100 Hz). (e) Tetanic stimulation induces rapid spine volume increase (red curve) and delayed increase in total  $\alpha$ CaMKII (green curve) in the stimulated spines ( $n = 4$  spines, 4 cells). (f) Tetanic stimulation leads to an increase in the total number of soluble and bound  $\alpha$ CaMKII molecules in the stimulated spines (2.0-fold change in bound  $\alpha$ CaMKII, 1.5-fold change in soluble  $\alpha$ CaMKII). Similar changes were induced by optical pairing (cf. Fig. 4). (g) Tetanic stimulation does not significantly change the fraction of bound  $\alpha$ CaMKII.