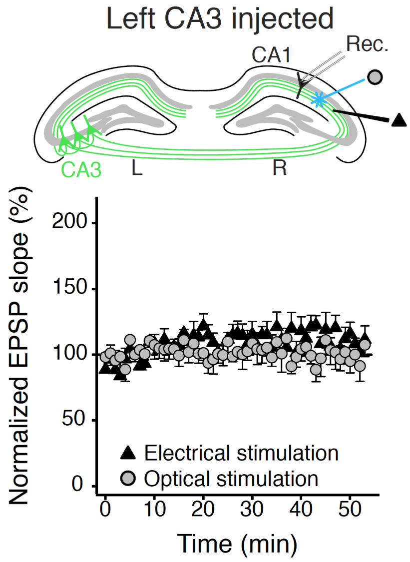
Supplementary Information

Hemisphere-specific optogenetic stimulation reveals left-right asymmetry of hippocampal plasticity

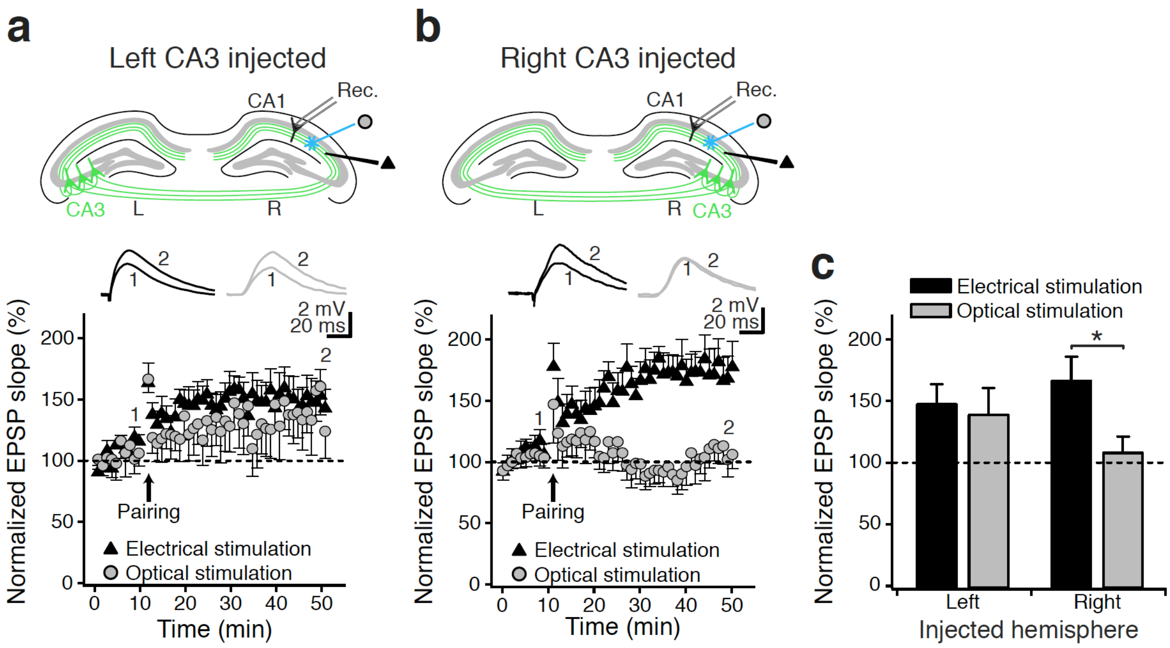
Michael M. Kohl, Olivia A. Shipton, Robert M. Deacon, J. Nicholas P. Rawlins, Karl Deisseroth, Ole Paulsen

Contents:

Supplementary Figures



Supplementary Figure 1 Stability of electrically and optically-evoked synaptic responses. Current-clamp recordings from CA1 pyramidal cells on the contralateral side of left-injected mice. Electrical stimulation (triangles) and optical stimulation (circles) were delivered in the stratum radiatum (n = 5). EPSPs stabilized 18–20 minutes after establishment of whole-cell configuration and remained stable for more than 50 minutes recording time. Error bars are s.e.m.



Supplementary Figure 2 Asymmetric induction of t-LTP at the CA3–CA1 pyramidal cell synapse with a strong pairing protocol. (**a, b**) With the experimenter blind to injection side, a stronger pairing paradigm consisting of 4 x 10 burst pairings at 5 Hz produced robust t-LTP in CA1 pyramidal neurons for electrical stimulation (triangles) in the stratum radiatum regardless of injection side. In contrast, hemisphere-selective optical stimulation of commissural fibers (circles) yielded robust t-LTP for left- but not right-injected mice. (**c**) Summary of results. Error bars are s.e.m. * $P < 0.05$, Student's *t*-test.

Supplementary Table 1   Paired-pulse ratios before and after t-LTP pairing protocol.

	Left				Right			
	Ipsi		Contra		Ipsi		Contra	
	Electric	*Light*	*Electric*	*Light*	*Electric*	*Light*	*Electric*	*Light*
n	6	6	5	5	6	5	5	5
Pre-pairing	1.9 ± 0.1	1.6 ±0.3	2.0 ± 0.5	1.6 ± 0.4	1.9 ± 0.2	1.7 ± 0.2	2.0 ± 0.1	1.9 ± 0.6
Post-pairing	2.0 ± 0.1	1.7 ± 0.1	1.8 ± 0.4	1.4 ± 0.4	1.9 ± 0.2	1.6 ± 0.3	1.9 ± 0.2	1.7 ± 0.3
% change	3.5 ± 6.8	16.6 ± 13.7	–6.6 ± 6.9	–15.7 ± 11.8	6.8 ± 9.6	–5.9 ± 7.9	–5.2 ± 4.0	7.2 ± 19.2

Supplementary Materials & Methods

Animals and AAV vectors

All procedures were carried out in accordance with British Home Office regulations. *CamKIIα::cre* mice[16] were obtained from Jackson Laboratories. ChR2 was fused to the fluorescent protein eYFP and cloned in the antisense direction into pAAV-MCS (Stratagene) to create AAV DIO ChR2-eYFP (for a vector outline and sequence see http://www.optogenetics.org). ChR2-eYFP was flanked by a pair of canonical loxP sites and a pair of mutated lox2272 sites, which are inverted by Cre to enable transcription from the EF-1α promotor. A woodchuck hepatitis B virus post-transcriptional element was placed in the sense direction 5' of the poly(A). Adeno-associated viral particles of serotype 2 were produced by the Vector Core Facility at The University of North Carolina at Chapel Hill.

Virus injections

CamKIIα-cre mice (5–10 weeks old) were anesthetized with 2–4% isoflurane at 0.6–1.4 min^{-1}. A small craniotomy was made 1.5 mm anterior and 2.2 mm lateral (either left or right) from interaural zero. Through a small durotomy, 0.1 μl fluorescent bead suspension (Molecular Probes) and 0.4 μl virus suspension (AAV DIO ChR2-eYFP, 4 x 10^{12} viral molecules ml^{-1}) were delivered at a rate of 0.1 μl min^{-1} 2.25 mm below the skull surface through a 33-gauge needle using a Hamilton Microliter syringe. Following a 5 minute wait after bolus injection, the needle was retracted by 0.2 mm and after another 5 minutes slowly retracted fully. The scalp incision was sutured, and post-injection analgesics and anti-inflammatory drugs (5 mg kg^{-1} meloxicam) were administered intraperitoneally to aid recovery.

Immunohistochemistry

Mice were anesthetized by intraperitoneal injection of 100 μl ketamine (100 mg ml^{-1}; Fort Dodge) and 50 μl medetomidin (1 mg ml^{-1}; Pfizer) and then transcardially perfused with phosphate-buffered saline (PBS, pH 7.4) containing 4% (wv^{-1}) paraformaldehyde (PFA) and 0.2% (wv^{-1}) picric acid. Brains were post-fixed for 24 hours at 4 °C in perfusion solution and subsequently infiltrated with 30% (wv^{-1}) sucrose in PBS for at least 24 hours. Coronal sections of 60 μm thickness were cut using a Leica SM 2000R sliding microtome. Sections were rinsed three times in Tris-buffered saline (TBS, Sigma), three times in TBS containing 3% (wv^{-1}) Triton X-100 (TBS-T), and once for 1 hour in TBS-T containing 20% (vv^{-1}) horse serum (Vector Labs) and then incubated for 48 hours at 4 °C in TBS-T containing 1% horse serum and anti-GFP (rabbit, 1:500, Sigma) and anti-Cre recombinase (mouse, 1:500, Millipore) antibodies, as well as 0.0001% DAPI (Sigma). The sections were rinsed four times in TBS and stained in TBS-T containing 1% horse serum and Alexa488- and Alexa546-labeled secondary antibodies (Invitrogen). After four rinses in TBS, slices were mounted in VectaShield (Vector Labs) and imaged on a Leica TCS SP2 confocal microscope.

Slice preparation and electrophysiology

Fourteen–28 days post-injection, mice were decapitated under deep isoflurane anesthesia and coronal slices of the hippocampal formation (350 μm) were prepared under low-light conditions. Slices were maintained submerged in oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF) containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose, pH 7.2-7.4. After recovering at room temperature (22-27 °C) for at least one hour, slices were transferred to the recording chamber and superfused with aCSF, bubbled with 95% O_2, 5% CO_2, at 1–2 ml min^{-1}. Cells with a pyramidal-shaped soma in the stratum pyramidale of CA1 were selected for recording using infrared, differential interference contrast optics[17].

Whole-cell patch-clamp recordings were performed with glass pipettes (3–5 MΩ for voltage-clamp, 5–7 MΩ for current-clamp), pulled from standard borosilicate glass. In voltage-clamp experiments the pipette solution contained (in mM): CsCl 140; EGTA 0.2; HEPES 10.0; ATP-Mg 2.0; GTP-NaCl 0.3; QX-314 5.0, adjusted to pH 7.2; osmolarity 280–290 mosmol l^{-1}). Voltage-clamp experiments were performed in the presence of gabazine (3 μM) with afferents from CA3 cut, and recordings were started after allowing at least 10 minutes for the intracellular solution to diffuse following breakthrough. Stimulation strength was adjusted to yield 150–200 pA EPSC peak amplitude at –90 mV.

In current-clamp experiments the pipette solution contained (in mM): 110 potassium-gluconate, 40 HEPES, 2 ATP-Mg, 0.3 GTP, 4 NaCl (pH 7.2–7.3; osmolarity 270–290 mosmol l^{-1}). In current-clamp recordings all cells were tested for regular spiking responses to positive current injection. Excitatory postsynaptic potentials (EPSPs) were evoked in the stratum radiatum at 0.1 or 0.2 Hz, alternating between an extracellular stainless steel electrode (20–60 μs, 150–350 μA) and a blue laser spot (473 nm, 5 ms, 1–5 mW at objective entry using a single mode fiber for laser-microscope coupling; UGA-40, Rapp OptoElectronic). Responses typically stabilized within 18–20 minutes, and then both electrical and optical pathway reliably produced stable EPSPs of equal magnitude over more than 35 minutes (**Supplementary Fig. 1**). To avoid intracellular wash-out we used only a 10 minute baseline after breakthrough and before pairing, and rejected recordings where the average EPSP slope for early (t = 0 to 3 min) and late (t = 7 to 10 min) baseline changed by > 10%. Two pairing protocols were used. One protocol consisted of simultaneous electrical and optical stimulation followed 5–10 ms later by a postsynaptic burst of action potentials and was repeated 100 times at baseline frequency. The other consisted of pairing simultaneous electrical and optical stimulation followed 5–10 ms later by a postsynaptic burst of action potentials 10 times at 5 Hz and repeating this train four times at 10 s intervals[8]. Data were low-pass filtered at 2 kHz and acquired at 5 kHz with a Molecular Devices Axon Multiclamp 700B amplifier (current clamp in bridge-mode). The signal was recorded on a PC using the Axon pClamp 9 acquisition software. Cells were rejected if series resistance changed by more than 20% during the course of the experiment. Data for t-LTP induced by the theta burst induction protocol (**Supplementary Fig. 2**) and all voltage-clamp experiments were collected and analyzed blind to injection side.

Data analysis

All analysis was performed using custom-made procedures in Igor Pro (Wavemetrics, OR, USA). Plasticity was assessed from the slope of the EPSP, measured on the rising phase of the monosynaptic EPSP as a linear fit between time points corresponding to 20% and 80% of the peak amplitude during baseline conditions. For statistical comparisons, the mean EPSP slope was averaged over the three minutes immediately before pairing and 27–30 minutes after pairing. Paired-pulse ratios were obtained from 6 consecutive recordings just before pairing and 30 minutes after pairing. The NMDA/AMPA receptor-mediated current ratio (NMDA/AMPA ratio) was calculated as described previously[14]. In brief, the AMPA receptor-mediated peak current was measured at a holding potential of –90 mV. The NMDA receptor-mediated current was estimated as the average current amplitude at a holding potential of +60 mV between 50 ms and 60 ms after the peak AMPA current. The NMDA/AMPA ratio was calculated by dividing the NMDA current estimate by the peak AMPA current at –90 mV. The liquid-junction potential was not compensated for. Representative traces are averages of 10 (voltage-clamp) or 5 (current-clamp) consecutive recordings. Statistical comparisons were made using one- and two-sample Student's *t*-test as appropriate. P-values less than 0.05 were considered significant. Data are presented as mean ± s.e.m.

Supplementary References

16. Hinds, H.L., Goussakov, I., Nakazawa, K., Tonegawa, S. & Bolshakov, V.Y. *Proc Natl Acad Sci USA* **100**, 4275-4280 (2003).

17. Stuart, G.J., Dodt, H.U. & Sakmann, B. *Pflugers Arch* **423**, 511-518 (1993).