Supplementary Methods

Slice Preparation Rat organotypic hippocampal slice cultures were prepared using methods similar to those described previously[16]. Briefly, 350 µm thick hippocampal slices were cut from 7 day old male Wistar rats, placed onto Millicell-CM membranes and maintained in culture media containing 25 % EBSS, 50 % MEM, 25 % heat-inactivated horse serum, glucose, and B27 (Invitrogen). Neurons were biolistically transfected after 5-6 days in vitro using a Helios Gene Gun in accordance with the manufacturer's instructions (Bio-Rad). The target DNA was either pLenti-hSyn-eNpHR3.0-EYFP (eNpHR3.0 fused to EYFP and driven by the human synapsin I promoter, generously provided by the Deisseroth lab, Stanford University) or FCK-Arch-GFP (Arch fused to GFP and driven by the CamKII promoter, generously provided by the Boyden lab, Massachusetts Institute of Technology). Recordings were performed 2-4 days post-transfection, which is equivalent to postnatal day 14-17. Previous work in rat hippocampus has shown that E_{GABAA} reaches mature levels within the first two postnatal weeks[17] and recordings from rat organotypic hippocampal slices have confirmed that GABAergic[18] and glutamatergic[19] synaptic transmission are mature at these stages(**Supplementary Fig. 1 and 2**).

Acute slices were prepared from 3- to 5-week-old male Wistar rats and 5- to 8-week-old CAMKII-cre mice (Jackson laboratory). Adeno associated virus serotype 2 (AAV2) carrying fusions for eNpHR3.0 and EYFP, or Arch and GFP, were injected into the hippocampus of the CAMKII-cre mice between postnatal days 14 and 21. Typical coordinates from Bregma for injections in ventral hippocampus were lateral, 3.1 mm; posterior, 2.7 mm; and 3.25 - 2.25 mm ventral to the surface of brain. Viral DNA included the double-floxed sequence for eNpHR3.0-EYFP driven by the elongation factor 1 promoter or the double-floxed sequence for Arch-GFP driven by the CAG promoter. Typical titers were ~10^{12} IU/ml. Injection volumes were 500 nl. After allowing 2 - 4 weeks for expression, acute horizontal hippocampal slices (350-400 µm thickness) were prepared.

The DNA constructs used to drive expression of the optical silencers were selected because they achieve good levels of neuronal expression, without toxicity effects, and are widely used within the field[9,10,20,21]. The experimental design compared the silencers by matching for photocurrent amplitude, which equates to the strength of the optical silencing, and therefore controlled for functional expression levels. In addition, there was no significant difference across the constructs in terms of the maximum photocurrent evoked (P = 0.47, ANOVA; maximum evoked photocurrent was 236.4 +/- 25.5 pA, 244.7 +/- 49.6 pA, 167.8 +/- 26.0 pA and 192.9 +/- 58.5 pA for NpHR in organotypics, Arch in organotypics, NpHR in acutes and Arch in acutes, respectively). Similarly, there was no significant difference across constructs in terms of resting E_{GABAA}, indicating that endogenous ion homeostasis mechanisms were comparable (P = 0.64, ANOVA; resting E_{GABAA} was -67.3 +/- 1.7 mV, -68.4 +/- 1.2 mV, -69.8 +/- 1.8 mV and -69.5 +/- 1.2 mV for NpHR in organotypics, Arch in organotypics, NpHR in acutes, and Arch in acutes, respectively).

Electrophysiology Hippocampal slices were transferred to the recording chamber and continuously superfused with 95 % O_2-5 % CO_2 oxygenated ACSF, heated to 30 °C[22]. For cell attached recordings 2-chloroadenosine (2 µM) was added to the ACSF to reduce spontaneous activity and for perforated patch recordings glutamatergic receptors and $GABA_B$Rs were blocked with kynurenic acid (2 mM) and CGP55845 (5 µM), respectively. Neurons within the pyramidal cell layer of the CA1 and CA3 regions of the hippocampus were targeted for recording. For cell-attached recordings pipettes (3-7 MΩ tip) were back-filled with an internal solution composed of (in mM): K-gluconate (130), NaCl (10), CaCl (0.1333), $MgCl_2$ (2), EGTA (1), KCl (4), and HEPES (10). For gramicidin perforated patch recordings, pipettes were filled with a high KCl internal solution whose composition was (in mM): KCl (135), Na_2ATP (4), 0.3 Na_3GTP (0.3), $MgCl_2$ (2), and HEPES (10). Gramicidin (Calbiochem) was dissolved in dimethylsulfoxide to achieve a stock solution of 4 mg/ml. Fresh stock solution was prepared daily and diluted in internal solution immediately prior to experimentation to achieve a final concentration of 80 µg/ml. The osmolarity of internal solutions was adjusted to 290 mOsM and the pH was adjusted to 7.38 with KOH.

Spike probability was assessed from recordings in the loose cell-attached patch configuration (50-150 MΩ). Synaptically-evoked spikes were triggered via a bipolar stimulating electrode placed in stratum radiatum, 300-400 µm from the recorded cell[23]. Stimulus intensity was set such that spikes (detected during a 200 ms window immediately after the stimulus) were evoked with a probability of approximately 0.4 before laser-activation (i.e. 4 out of 10 trials resulted in at least one spike). The 'before laser' stimulus was delivered 1250 ms before laser onset and the 'after laser' stimulus was delivered 250 ms after laser offset. We tested for post-inhibitory rebound spikes by examining responses to 15 s of laser-activation, in the absence of a synaptic stimulus. Consistent with previous reports[24], cells showing rebound spikes were rare (7 out of 54) and were not included in the analyses. Perforated patch recordings were started once the access resistance had stabilized between 20-50 MΩ (mean R_a ~ 35 MΩ). For all experiments online series resistance correction of 70 % was used. Recordings were made using an Axopatch 700A amplifier and data acquired using Clampex software (Molecular Devices). All values reported from voltage clamp recordings were corrected offline for the liquid junction potential (4.2 mV) between the intracellular and extracellular solution.

$GABA_A$Rs were activated by delivering short 'puffs' of GABA (100 µM) in the presence of glutamate receptor blockers and $GABA_B$R blockers (see above). The agonist was applied via a patch pipette positioned close to the soma and connected to a picospritzer. To calculate resting E_{GABAA} and g_{GABAA}, $GABA_A$R currents were measured at five different holding potentials (5 mV intervals around the resting membrane potential) in response to a GABA puff. A minimum of 30 s was allowed before each puff in order to allow full recovery of Cl^- homeostasis[13,14,25]. The peak $GABA_A$R current was plotted as a function of holding potential to generate a current–voltage curve (**Fig. 2c**), from which resting E_{GABAA} was defined as the *x*-intercept value and the peak $GABA_A$R conductance (g_{GABAA}) as the slope. To measure the impact of photocurrents on E_{GABAA}, it was important to estimate E_{GABAA} from single $GABA_A$R currents. To achieve this, resting E_{GABAA} and g_{GABAA} were calculated before each experiment (as described above) and these values were

then used to estimate E_{GABAA} for a single $GABA_AR$ current by assuming a constant g_{GABAA} across GABA puffs and solving the equation:
$GABA_AR$ current = g_{GABAA} (Holding potential - E_{GABAA}).

The GABA puffs enabled us to consistently activate $GABA_ARs$ on the somatic compartment of the recorded neuron, where the perforated patch clamp recordings have the best control of membrane potential, thus optimising our measurements of E_{GABAA}. The peak g_{GABAA} was not statistically different between NpHR-expressing cells (9.70 ± 1.69 nS, N=26) and Arch-expressing cells (9.27 ± 1.76 N=21, P = 0.86, t test). In addition, there was no correlation between the peak g_{GABAA} and the photocurrent induced shift in E_{GABAA} for either NpHR-expressing cells (r = 0.02, P = 0.90, Pearson Correlation) or Arch-expressing cells (r = 0.03, P = 0.89, Pearson Correlation).

Data analysis was performed using custom-made programs in the MATLAB environment (MathWorks). Some statistical analysis was also performed using GraphPad Prism version 5.00 (GraphPad Software). Data are reported as mean ± SEM.

Photoactivation of NpHR and Arch was achieved via a diode-pumped solid state (DPSS; 532 nm peak wavelength) laser (Shanghai Laser Optic Century). The laser was coupled to a 1000 μm diameter mulitimode optic fiber via a collimating lens (Thorlabs). The tip of the optic fiber was positioned at an image plane within the microscope in the center of the optical axis, and directed into the objective lens via a dichroic mirror. This resulted in a spot of light at the brain slice whose diameter was 195 μm, assuming zero tissue scattering. Laser stimulation elicited photocurrents in both Arch- and NpHR- expressing cells of comparable size (**Supplementary Fig. 3c**) and kinetics (**Supplementary Fig. 3a,b**) to those reported previously[9,11]. In agreement with published work, both constructs appeared to have no observable toxic effects on the tissue concerned[9,11]. For the gramicidin recordings, functionally relevant laser intensities were defined by assessing the ability of the photocurrents to inhibit spiking in response to somatic current injections via the recording pipette (see **Fig. 2**). First, we injected a range of current steps in current clamp (1 s duration) without any laser-activation, from which we defined a 'threshold' somatic current (the minimum current that evoked spiking; mean of 112 ± 25 pA, which generated a mean spike rate of 4.1 ± 0.8 Hz, n = 12) and a 'strong' somatic current (twice the amplitude of the threshold current; mean of 224 ± 50 pA, which generated a mean spike rate of 13.3 ± 2.6 Hz). An 'intermediate' laser intensity produced the minimum mean photocurrent (104 ± 17 pA, range 40 - 230 pA) required to inhibit all spiking activity in response to the threshold somatic current. A 'higher' laser intensity produced the minimum mean photocurrent (207 ± 35 pA, range 90 - 400 pA) required to inhibit all spiking in response to the strong somatic current injection.

References for Methods

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