Online Methods

Animals. A total of 30 male adult mice (age 5-10 weeks, body weight 30-40g) were used in this study. Animals were kept on a 12h:12h light/dark cycle and provided with ad libitum access to food and water. Among 14 transgenic mice expressing the Cre-recombinase under the control of the Parvalbumin promoter (PV-Cre, Jackson Laboratory, B6;129P2-Pvalbtm1(cre)Arbr/J), 9 were injected with Cre-dependant Channelrhodopsin2 (ChR2) for optogenetic activation of PV interneurons with light pulses delivered from a laser source through an optic fiber, and 5 with the Cre-dependant Pharmacologically Selective Actuator Module fused to the glycine receptor (PSAM-GlyR) for pharmacogenetic silencing of PV-interneurons with the exogenous specific ligand PSEM-89S[1, 2]. Among 16 wild-type mice (B6129SF2), 8 received an intra-hippocampal injection of the convulsive agent kainic acid (Sigma-Aldrich Co., St Luis, MO), and the other 8 served as controls. All experimental procedures were performed in accordance with the EU directives regarding the protection of animals used for experimental and scientific purposes (86/609/EEC and 2010/63/EU), with the French law, and approved by the Ethical committee CEEA50 (saisines #10894, 10896 and 19354).

Surgical procedures. The mice were anesthetized with isoflurane (1.5 - 5%), placed in a stereotaxic frame (David Kopf Instruments) and kept on a thermal blanket (Physitemp) to maintain their body temperature at 37.5°C. The eyes were covered with a protective liquid gel (Ocrygel). The scalp and periosteum over the dorsal surface of the skull were removed. **For virus injection** (optogenetic and pharmacogenetic constructions), a small craniotomy was executed at the coordinates of the left dorsal hippocampus (AP-1.6mm and L2.5mm relative to Bregma), and the injections (500nl measured using a Hamilton syringe, 2mm deep from the brain surface) performed (n = 14 PV-Cre mice) using a thin glass capillary (40-80µm at the tip). After injection, the capillary was maintained *in situ* for about 10 min before being slowly removed. The scalp was then sutured and the animal housed individually for 3-6 weeks before electrophysiological recording. **For head-restrained experiments** (n = 4 PV-Cre mice), reference and ground wires were implanted in the cerebellum and attached to a 2-pin connector, maintained together with a small custom-made horizontal stainless-steel bar anchored to the skull above the cerebellum with dental acrylic. A thin anchoring layer of dental acrylic (Super-Bond, Frapident) was applied on the exposed skull, except at specific locations in which a thin layer of bone was kept intact to allow for dorsal CA3 targeting with the recording silicon probe (left hemisphere, AP-1.6, L2.5, vertical insertion) and optical fiber (AP-1.6, L 3.2, insertion with an angle of 15° from vertical) as well as contralateral (right hemisphere) dorsal hippocampus targetting of the capillary for bicuculline injection (AP-1.6 L1.5, insertion 20° from vertical). **For the study of chronic epileptogenesis** (n=8 WT mice), a craniotomy was performed above the dorsal hippocampus (right hemisphere, AP-

1.6, L1.5) for vertical insertion of a capillary and injection of kainic acid (0, 2µg dissolved in saline, 500nl measured using a Hamilton syringe) at 1.8 mm from the brain surface. After injection, the capillary was maintained *in situ* for an additional 5 min before being slowly removed. The scalp was then sutured and the animal housed individually for 1 week before electrophysiological recording, during the latent period that precedes the development of chronic epilepsy[3, 4]. Bicuculline, diazepam and kaïnic acid were purchased from Sigma-Aldrich Co., St Luis, MO.

Optogenetic excitation and chemogenetic inhibition of PV interneurons. For **optogenetic** excitation of PV interneurons, PV-Cre mice were injected with an adeno-associated virus (AAV) bearing ChR-2 with a YFP reporter, pAAV-Ef1a-DIO hChR2 (E123T/T159C)-EYFP (gift from Karl Deisseroth, packaged into AAV serotype 9 from Addgene, plasmid # 35509, initial viral titer ≥1x10*13 gcp/ml, diluted four times in HBSS). Optogenetic activation (light wavelength 473nm, power max 10-14mW, 2ms pulses, 0.5Hz, driven by a train generator and current stimulator from Digitimer Ltd) was delivered through an optical fiber (diameter 200µm) connected to a diode-pumped solid-state (DPSS) laser (Shangai instrument, 100mW or IKECOOL Corporation). For **pharmacogenetic** inhibition of PV interneurons, we used an AAV bearing PSAM-GlyR with a GFP reporter, -rAAV-syn::FLEX-rev::PSAML141F,Y115F:GlyR-IRES-GFP (Addgene, gift from Scott Sternson, plasmid # 32481, packaged into AAV serotype 9, initial viral titer ≥10*13 gcp/ml, diluted four times in HBSS). PSAM-GlyR activation was performed by the injection of the specific ligand PSEM-89S (150-200nl, 15µM in saline, obtained from Scott Sternson, Janelia Farm Research Campus, VA, USA), pressure-ejected into the left hippocampus from a glass capillary (40-80µm at the tip).

Head-restrained recordings. The mice (n = 4 PV-Cre), previously implanted with head-posts, were maintained in the stereotaxic apparatus from their implanted fixation bars. Their body was inserted into a plastic tube (diameter approximately 5cm) to provide a reassuring confined environment and prevent excessive body movement. The animals readily habituated to head-fixed conditions. After initial training periods of a few minutes, restraint duration was gradually increased each day until the mouse would sit calmly for a period of roughly 1h. Electrophysiological recordings were performed after several days (at least five) of habituation. The dominant behavioral state in the head-restrained condition was awake immobility. On the day of recording, under brief isoflurane anesthesia (and body temperature control with a heating pad, 37.5°C), the craniotomies were completed and the silicon probe, optical fiber and injection-capillary inserted. After 1h recovery from anesthesia, and baseline recording, bicuculline was injected (100-200nl, 100µM in saline) into the right dorsal hippocampus to induce acute hippocampal seizures. At the end of the recording session, the animals were sacrificed and their brains removed for histological verification of electrode and optical fiber positions.

Multi-site extracellular recording in vivo. Extracellular recordings of spontaneous multi-unit activity and local field potentials were performed on mice either drug free in the head-restrained configuration (n = 4 PV-Cre mice) or anesthetized (n = 10 PV-Cre, 8 control and 8 KA-injected animals) with urethane (1.7g/kg, IP) and a complement of ketamine/xylazine (respectively 6.6 and 0.66mg/kg, IM, repeated whenever necessary, usually every 30 to 60min). Animals were fixed in the stereotaxic apparatus (David Kopf instrument), the craniotomy was performed, the dura was gently removed, and a multi-site silicon probe (Neuronexus Technologies, either A1x32-Poly3-5mm-25s-177, 32 channels arranged on a single shank as 3 rows of vertically arranged staggered recording sites, vertical separation 25µm, or Buzsaki16, 2 shanks separated by 200 µm, 8 recording sites each separated by 10-20µm), covered with DiI (Molecular Probes) for post-hoc verification of electrode position, was inserted vertically through the neocortex until the pyramidal layer of the CA3a hippocampal region. Recordings were performed using either Lynx-8 amplifiers (Neuralynx, gain x1000, bandpass filter 0.1Hz to 9KHz) and a digitizer (United Electronics, 14bits, A/D Gain x2, sampling rate 20KHz) controlled by a custom-made LabView (v7.1, National Instruments) program, or an integrated Digital-Lynx SX system (Neuralynx, Cheetah v5.0 software, 24bits, sampling rate 32KHz, bandpass 0.1Hz to 9KHz and range ±5mV for LFP analysis, as well as bandpass 1Hz to 9KHz and range ±1mV for spike sorting), and stored on a PC for offline analysis. The pyramidal layer was recognized electrophysiologically by the typical presence of fIPSPs and multi-unit firing. The recording started approximately 30 minutes after the insertion of the probe. Urethane was purchased from Sigma-Aldrich, ketamine (Imalgene 1000) from Merial and xylazine (Rompun 2%) from Bayer.

Histological Processing. At the end of the recording session, the animal was transcardially perfused with 4% paraformaldehyde. Coronal sections (60µm-thick, cut using a VT1000S Leica vibratome) were blocked in 5% Bovine Serum Albumin (BSA) and 0,25% Triton X-100. After Tris-buffered saline washes, sections were incubated overnight at 4°C in 1% BSA, 0,25% Triton X-100 with mouse primary antibody against parvalbumin (1:200, Sigma-Aldrich, Clone PARV-19 P3088) and rabbit antibody against GFP conjugated to Alexa Fluor 488 (1:2000, Thermo Fisher, #A-21311). After Tris-buffered saline washes, sections were then incubated with 1% BSA, 0,25% Triton X-100 and Alexa Fluor 647 conjugated goat antibody to mouse (1:500, Thermo-Fisher) 4h at room temperature. After Tris-buffered saline washes, sections incubated 10 minutes with DAPI (1:1000 Sigma-Aldrich Co., St Luis, and MO) at room temperature. After washing, sections were mounted and coverslipped on slides. Confocal stack images from the CA3a hippocampal region were acquired. The number of GFP and PV-immunopositive cell bodies was counted on the stacks with the IMARIS software (Oxford instruments). To quantify co-

localization, individual GFP labeled interneurons were identified and subsequently scored for PV immunoreactivity. We collected scans from each sample in multiple slices.

Data analysis. Data were visualized and processed using NeuroScope and NDManager from the Neurosuite software[5] (http://neurosuite.sourceforge.net), and analyzed using Excel (Microsoft Office 2013), Origin (OriginLab, Northampton, MA) and MATLAB (MathWorks) built-in or custom-built procedures. The spikes were detected and extracted (SpikeDetekt), automatically clustered (KlustaKwik), and the resulting clusters manually verified and refined (KlustaViewa) using the KlustaSuite software[6] (www. cortexlab.net/tools). Beside multi-unit activity (MUA), only the clusters with a clear refractory period and typical bursting pattern of putative pyramidal cells were included in the analysis of neuronal firing (and referred to as pyramidal cells). The fIPSPs were detected with the MiniAnalysis software (6.0.3 Synaptosoft Inc.) from the raw LFP using the following parameters: amplitude >100μV, rise time 1 to 6ms (anesthetized) or 1 to 3ms (head-fixed), decay 3 to 10ms (anesthetized) or 1 to 5ms (head-fixed), halfwidth 2 to 8ms (anesthetized) or 2 to 6ms (head-fixed), and decay10-90-slope 100 to 1500 μV/ms (anesthetized) or 100 to 500 μV/ms (head-fixed). Epileptiform events (inter-ictal spikes) were identified with Minianalysis as events of positive polarity and larger than 5 times the average amplitude of the detected fIPSPs. All detected events were verified by visual inspection. Peri-event time histograms display the time-distributions of neuronal activity relative to fIPSPs detected from the same electrode/shank. The number of spikes was counted within time-bins of 1ms around reference events (either fIPSP-peak or optogenetic light-stimulation onset), and normalized by the total number of spikes within the histogram. Baseline activity was computed as the mean (and SD) of values between -50 and -30ms. Pyramidal cells were considered as inhibited by fIPSPs if their firing rate decreased and remained below that of [baseline - 2SD] from 0 to at least 4ms after fIPSP-peak. The strength of inhibition was then quantified as the percentage of firing inhibition (relative to baseline) in the inhibitory trough (bin of minimum firing in the 4ms post-fIPSPs), and the duration of recovery as the latency to the last of post-fIPSPs time-bin below [baseline - 2SD]. Only cells with [baseline - 2SD] > 0 were considered to have high and stable enough firing for the possible detection of potential fIPSP-mediated inhibition. Pyramidal cells were considered as excited by fIPSPs if their firing rate increased above [baseline + 3SD] within 4ms after fIPSP-peak. Because bursts of action potentials (short interspike intervals) which first spike would evoke a fIPSP would potentially produce a spurious post-fIPSP peak in the peri-event histogram that may be wrongly interpreted as pyramidal cell excitation, we also computed the peri-event histograms of putatively excited pyramidal cells excluding all the spikes preceded by an action potential by less than 10ms. The post-fIPSP peak-firing was still present in all the tested putatively excited pyramidal cells, excluding the contribution of misinterpretation due to burst firing. Pyramidal cells were considered as non-responding if they were

neither inhibited nor excited by fIPSPs. All statistical analyses were performed in Matlab (MathWorks) or Origin (OriginLab). No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to those generally employed in the field. All tests were two-tailed unless otherwise indicated. Our data did not follow normal distributions, therefore the non parametric Wilcoxon Mann-Whitney test was used. Results are displayed as mean ± SE unless indicated otherwise.

References

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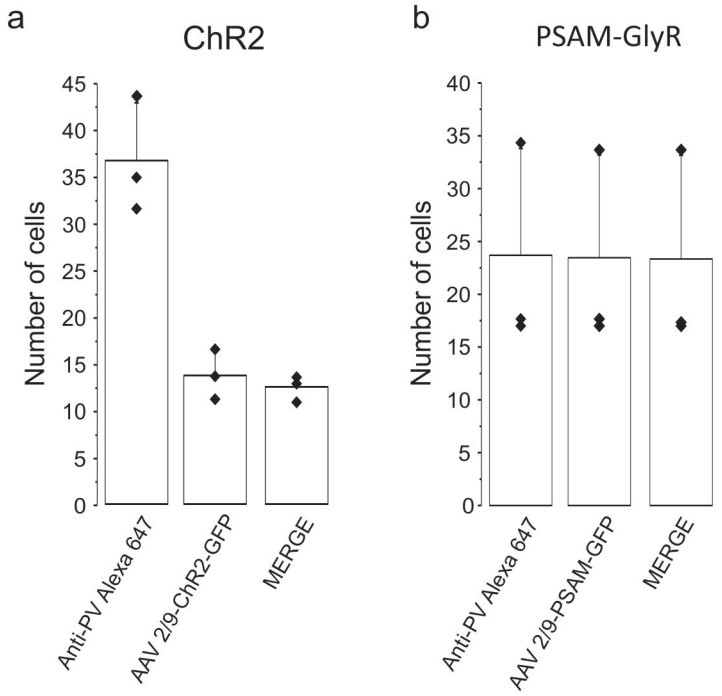
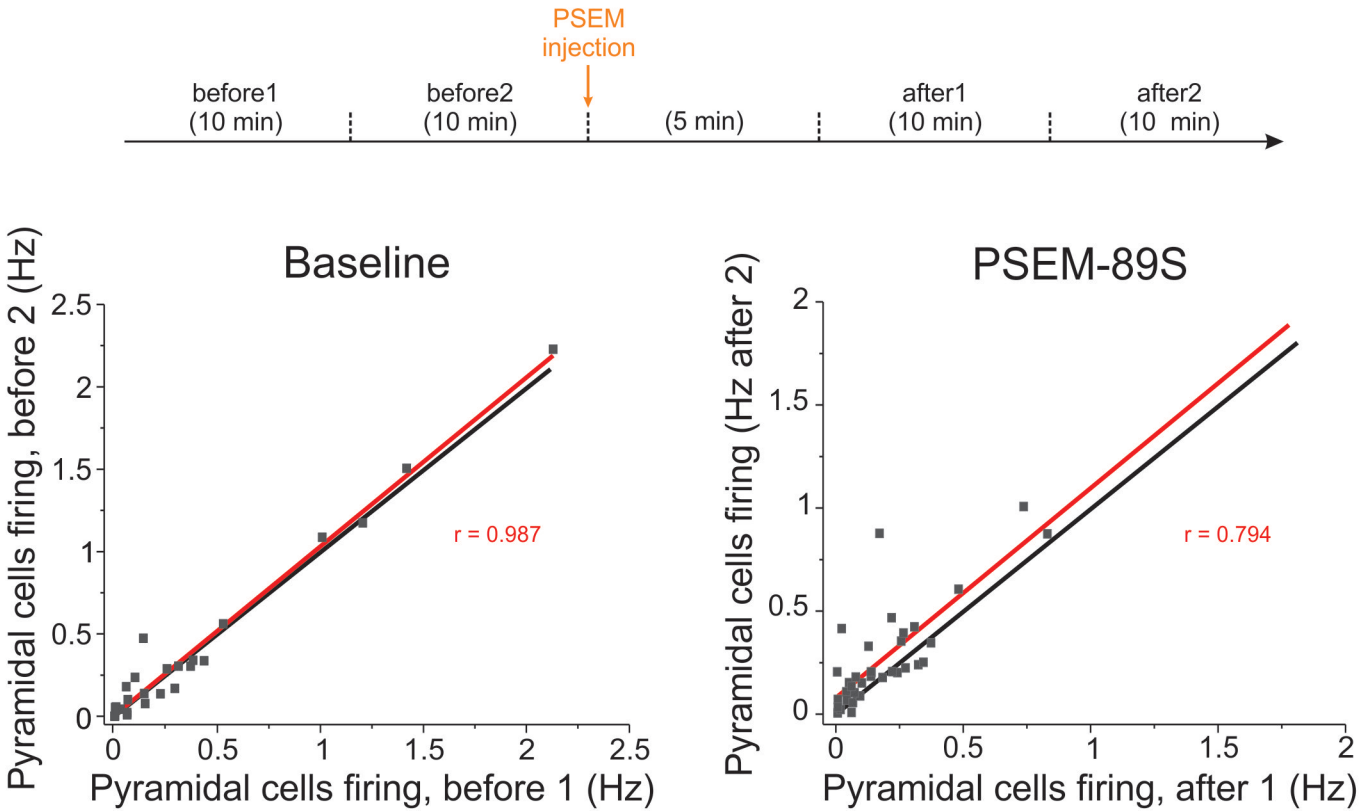


Supplementary Figure 1: **Immuno-histological control of ChR2 and PSAM-GlyR expression in PV interneurons**

(a) Summary plot (mean ± SD, n=3 mice) of the number of cells immuno-positive for PV (anti-PV Alexa 647), for ChR2-GFP, and for both (merge). Note that only a fraction of PV cells express ChR2 but that virtually all cells expressing ChR2 are PV cells, indicative of high specificity of ChR2 expression for PV cells.

(b) Summary plot (mean ± SD, n=3 mice) of the number of cells immuno-positive for PV (anti-PV Alexa 647), for PSAM-GlyR-GFP, and for both (merge). Note that almost all PV cells also express PSAM-GlyR and that virtually all cells expressing PSAM-GlyR are PV cells, indicating high specificity and high coverage of infection for PV cells.



Supplementary Figure 2: Redistribution of pyramidal cells firing rates after pharmacogenetic blockade of PV interneurons

Comparison of discharge frequency of individual pyramidal cells (n = 36 neurons from 3 mice) in two successive recordings of 10min, either both before (left, **baseline**) or both after (middle, **PSEM-89S**) the injection of PSEM-89S, as depicted in the **timeline on top**. **Black line**, bisector (y=x). **Red line**, linear regression line. Note high correlation values (**r**) in both baseline and PSEM-89S, indicating stable activity among CA3 pyramidal cells.