Parvalbumin and somatostatin interneurons contribute to the
generation of hippocampal gamma oscillations

Pantelis Antonoudiou¹, Yu Lin Tan¹, Georgina Kontou³, A. Louise Upton¹,² and Edward O. Mann¹,²
¹Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, OX1 3PT, UK
²Oxford Ion Channel Initiative, University of Oxford, OX1 3PT, Oxford, UK
³Neuroscience, Physiology and Pharmacology, University College London

Corresponding author’s electronic address: ed.mann@dpag.ox.ac.uk

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Abstract

Gamma-frequency oscillations (30-120 Hz) in cortical networks influence neuronal encoding and information transfer, and are disrupted in multiple brain disorders. While synaptic inhibition is important for synchronization across the gamma-frequency range, the role of distinct interneuronal subtypes in slow (< 60 Hz) and fast gamma states remains unclear. Here, we used optogenetics to examine the involvement of parvalbumin (PV+) and somatostatin (SST+) expressing interneurons in gamma oscillations in the mouse hippocampal CA3 ex vivo, using animals of either sex. Disrupting either PV+ or SST+ interneuron activity, via either photo-inhibition or photo-excitation, led to a decrease in the power of cholinergically-induced slow gamma oscillations. Furthermore, photo-excitation of SST+ interneurons induced fast gamma oscillations, which depended on both synaptic excitation and inhibition. Our findings support a critical role for both PV+ and SST+ interneurons in slow hippocampal gamma oscillations, and further suggest that intense activation of SST+ interneurons can enable the CA3 circuit to generate fast gamma oscillations.

Significance statement: The generation of hippocampal gamma oscillations depends on synchronised inhibition provided by GABAergic interneurons. Parvalbumin expressing (PV+) interneurons are thought to play the key role in coordinating the spike timing of excitatory pyramidal neurons, but the role distinct inhibitory circuits in network synchronisation remains unresolved. Here, we show for the first time that causal disruption of either PV+ or somatostatin expressing (SST+) interneuron activity impairs the generation of slow gamma oscillations in the ventral hippocampus ex vivo. We further show that SST+ interneuron activation along with general network excitation is sufficient to generate high frequency gamma-oscillations in the same preparation. These results affirm a crucial role for both PV+ and SST+ interneurons in hippocampal gamma oscillation generation.

Introduction

Gamma oscillations (30 - 120 Hz) are a common feature of active cortical networks, which have been proposed to contribute to local gain control (Sohal et al., 2009; Cardin et al., 2009; Sohal, 2016) and facilitate transmission between synchronised neuronal assemblies (Fries, 2005; Akam & Kullmann,
While the function of gamma oscillations remains debated (Burns, Xing & Shapley, 2011; Butler & Paulsen, 2014; Bastos, Vezoli & Fries, 2015; Ray & Maunsell, 2015; Womelsdorf & Everling, 2015; Lasztóczi & Klausberger, 2016; Sohal, 2016), changes in these rhythms continue to act as a useful marker of function and dysfunction in cortical circuit operations (Bragin et al., 1995; Fries et al., 2001; Herrmann & Demiralp, 2005; Uhlhaas & Singer, 2006; Basar-Eroglu et al., 2007; Uhlhaas & Singer, 2010; Yamamoto et al., 2014; Spellman et al., 2015). There is a general consensus that the generation of gamma rhythms depends upon the spiking of inhibitory interneurons, which synchronise the firing of excitatory pyramidal cells via fast synaptic inhibition (Whittington, Traub & Jefferys, 1995; Penttonen et al., 1998; Csicsvari et al., 2003; Hájos et al., 2004; Mann et al., 2005; Hasenstaub et al., 2005; Bartos, Vida & Jonas, 2007; Buzsáki & Wang, 2012; Kim et al., 2016; Chen et al., 2017; Veit et al., 2017). Specifically, parvalbumin-expressing (PV+) interneurons, which target the perisomatic domain of pyramidal neurons, are thought to play the key role in generating and maintaining gamma oscillations in the brain (Csicsvari et al., 2003; Hájos et al., 2004; Mann et al., 2005; Gloveli et al., 2005; Hájos & Paulsen, 2009; Tukker et al., 2013; Cardin, 2016; Penttonen et al., 1998). PV+ interneurons are adapted for fast synchronisation of network activity, as they resonate at gamma frequencies and exert strong perisomatic inhibition that is capable of precisely controlling spike timing (Pike et al., 2000; Pouille & Scanziani, 2001; Cardin et al., 2009; Bartos & Elgueta, 2012; Hu, Gan & Jonas, 2014; Kohus et al., 2016).

Recently, a selective role for PV+ interneurons in gamma-frequency synchronisation has been challenged by several studies performed in the primary visual cortex (Chen et al., 2017; Veit et al., 2017; Hakim, Shamardani & Adesnik, 2018). In this brain region, it was shown that dendrite-targeting somatostatin-expressing (SST+) interneurons were the main contributors for the generation of slow gamma oscillations, while PV+ interneurons were more important for higher frequency synchronisation (Chen et al., 2017). Previous studies have found analogous roles for SST+ and PV+ interneurons in low- and high-frequency network synchronisation (Beierlein, Gibson &
Connors, 2000; Gloveli et al., 2005; Tukker et al., 2007; Craig & McBain, 2015). However, it is not yet clear whether SST+ interneurons might play a more generic role in the generation of slow gamma oscillations across brain areas.

The hippocampus displays both slow and fast gamma rhythms during theta activity, with slow gamma generated in CA3 and fast gamma propagated from entorhinal cortex (Bragin et al., 1995; Colgin et al., 2009; Schomburg et al., 2014; Lasztóczi & Klausberger, 2016). The circuitry for slow gamma oscillations is preserved in hippocampal slices (Fisahn et al., 1998), and these models have been used extensively to show that PV+ interneurons are strongly phase-coupled to gamma oscillations, and contribute to rhythmogenesis (Hájos et al., 2004; Mann et al., 2005; Gloveli et al., 2005; Gulyás et al., 2010). However, the majority of interneurons are phase-coupled to ongoing slow gamma oscillations (Hájos et al., 2004; Gloveli et al., 2005; Oren et al., 2006), and it may be that SST+ interneurons play an important role in synchronising PV+ networks. Indeed, whether specific classes of CA3 interneuron are necessary and sufficient for the generation of slow gamma oscillations has not yet been tested. Here, we took advantage of optogenetic techniques (Nagel et al., 2003; Chow et al., 2010; Boyden et al., 2005) to test the involvement of PV+ and SST+ interneurons in cholinergically-induced gamma oscillations in the CA3 of acute hippocampal slices.

**Materials and Methods**

**Transgenic mice**

All procedures were performed according to the United Kingdom Animals Scientific Procedures Act (ASPA) 1986 and the University of Oxford guidelines. Adult (older than 8 weeks, both male and female) PV-cre (B6;129P2-Pvalbtm1(cre)Arbr/J), PV-cre-Ai9 (PV-Cre x Gt ROSA (CAG-tdTomato) Hze/J), and SST-cre mice (Sst tm2.1(cre)Zjh/J) were used for all experiments.
**Stereotaxic viral injections**

Anaesthesia was induced in mice with 4 % isoflurane/medical oxygen mixture (2 L per min). The area around the head was shaved and cleaned in preparation for scalp incision. Anaesthesia was subsequently maintained using 1.5 - 2.5 % isoflurane at a rate of 2 L per min. Before the onset of the procedure a cocktail of systemic peri-operative analgesics (Metacam (Meloxicam) 1 mg/Kg and Vetergesic (Buprenorphine) 0.1 mg/Kg) and a local analgesic (Marcaine (Bupivacaine) 10mg/Kg) were administered subcutaneously (Oxford University Veterinary Services). Following, antibiotic solution was applied on the head and an incision of the scalp was performed that allowed a small craniotomy to be made. A 33/34-gauge needle was attached on a Hamilton Microliter Syringe and used to inject the virus solution at a rate of ≈100 nL/min (viral concentration ≈ 10^{12} genome copies per mL). After every injection, the needle was left stationary for at least three minutes to allow diffusion of virus in the surrounding area. The virus solution was injected with the aid of a stereotaxic frame into ventral CA3 area of hippocampus (2.7 mm caudal and 2.75 mm lateral from Bregma). A total of 600 - 800 nL were injected at two depths (300 - 400 nL at 3.1 mm and 300 - 400 nL at 2.7 mm). Following the injection, local analgesic (Marcaine 10 mg/Kg) was applied on the incised scalp before it was sutured. The animals were then transferred in a heating chamber and allowed to recover. The animals were monitored, and welfare scored in the following days to ensure that they properly recovered after surgery. Injected mice were assessed for viral expression after a minimum of 3 weeks. All viral constructs were acquired from Vector Core Facilities, Gene Therapy Centre (North Carolina, UNC). Viral constructs used: AAV5-EF1a-DIO-ChR2(H134R)-mCherry, AAV5-EF1a-DIO-ChR2(H134R)-eYFP, AAV-EF1a-DIO-Arch3.0-EYFP, AAV-Ef1a-DIO-hChR2(E123T-T159C)-p2A-mCherry-WPRE (Dr. Karl Deisseroth), and AAV-CAG-FLEX-ArchT-GFP (Dr. Ed Boyden).

**Ex vivo brain slice preparation**
Mice were anaesthetised using 4 % isoflurane (Oxford University Veterinary Services) and were sacrificed by decapitation after the pedal reflex was abolished. Brains were extracted in warm (30 - 35 °C) sucrose solution (34.5 mM NaCl, 3 mM KCl, 7.4 mM MgSO₄·7H₂O, 150 mM sucrose, 1 mM CaCl₂, 1.25 mM NaH₂PO₄, 25 mM NaHCO₃ and 15 mM glucose) and transverse hippocampal slices of 350 μm thickness were cut using a Leica vibratome (VT 1200S) (Huang et al. 2013). Slices were then immediately placed in an interface storing chamber containing warm (30 - 35 °C) aCSF (126 mM NaCl, 3.5 mM KCl, 2 mM MgSO₄·7H₂O, 1.25 mM NaH₂PO₄, 24 mM NaHCO₃, 2 mM CaCl₂ and 10 mM glucose) at least one hour to equilibrate. All solutions were bubbled with 95% O₂ and 5% CO₂ beginning 30 minutes before the procedure until the end of the experiment.

**Electrophysiology**

Extracellular recordings were conducted in an interface recording chamber at 33-34 °C. Visualisation of the slices and electrode placement was performed using a Wild Heerbrugg dissection microscope. Local field potentials were recorded by inserting a borosilicate glass electrode filled with aCSF (tip resistance = 1 - 5 MΩ) in CA3 pyramidal layer. Data were acquired and amplified (x 10) by Axoclamp 2A (Molecular Devices). The signal was further amplified x 100 and low pass filtered at 1 KHz (LPBF-48DG, NPI Electronic). The signal was then digitised at 5 kHz by a data acquisition board (ITC-16, InstruTECH) and recorded from the IgorPro (Wavemetrics). Gamma oscillations were induced by the application of 5 μM carbachol (Cch). The LFP signal was quantified using real-time fast Fourier transform (FFT) analysis and oscillations were detected by a peak in the power spectrum at low - band frequencies (25 - 49 Hz). For unit recordings a linear 16 channel tungsten multi-electrode array (MEA; MicroProbes) was lowered in the CA3 subfield. The array channels had 100 μm spacing to ensure full coverage of the hippocampus. The MEA was mounted on an RHD2132 Amplifier board and connected to the RHD2000 USB Interface Board (Intan Technologies). Data were acquired at a rate of 20 kHz using the RHD2000 rhythm software (Intan Technologies).
Intracellular recordings were always conducted in a single submerged chamber (26 - 32 °C) using borosilicate glass pipettes (5-12 MOhm). The signal was acquired through the MultiClamp 700B amplifier (Molecular Devices) and digitised at a rate of 10 kHz by a data acquisition board (ITC-18, InstruTECH) and was then recorded using the Igor Pro 6.37 software. The signals were low pass filtered (Bessel) at 10 kHz for current clamp mode and 3 kHz for voltage clamp (VC) mode. Slice and cell visualisation were achieved using oblique illumination and monitored through a HAMATATSU ORCA - ER digital camera. Filtered white LED (460 +/- 30 nm, 1.53 mW, Thor Labs) via epi-illumination was used to activate channelrhodopsin (ChR2). Filtered white LED (525 +/- 20 nm, 1.45 mW, Thor Labs) via epi-illumination was used to activate archaerhodopsin (Arch). For a power of 1.5 mW, the light intensity in the illuminated area was 3.68 mW / mm². Cell attached recordings were performed in current clamp (IC) mode (Multiclamp software) using glass pipettes filled with aCSF.

For whole cell current clamp recordings pipettes were filled with internal solution containing 110 mM KGlucnoate, 40 mM HEPES, 2 mM ATP-Mg, 0.3 mM GTP-NaCl, 4 mM NaCl, (3-4 mg/ml biocytin, Sigma). For whole cell voltage clamp recordings, pipettes were filled with internal solution containing 140 mM Cesium methanesulfonate, 5 mM NaCl, 10 mM HEPES, 0.2 mM EGTA, 2 mM ATP-Mg, 3 mM GTP-Na, 5 mM QX-314, (3-4 mg/ml biocytin). Series resistance compensation was not performed in all cells included for analysis. For perforated patch recordings the tip of the pipette was filled with a KCl-containing solution (150 mM KCl and 10mM HEPES, pH 7.2-7.3; Osmolality 300 mOsmol/Kg). The rest of the pipette was filled with the same KCl solution containing 5 μM gramicidin D (1:1000 DMSO dilution, Sigma) and 10 μM Fluorescein (Sigma) to visualise if there was spontaneous rupture of the membrane during patching experiments.

**Light delivery**

For photo-excitation (ChR2) experiments, light illumination was delivered through a fibre optic using a blue LED (470 +/- 20 nm, Thorlabs, M470F3; max power at fibre optic tip = 10 mW). For photo-
inhibition (Arch) experiments light illumination was delivered through a fibre optic by a green LED (530 +/- 30 nm, Thorlabs, M530F2; maximum power at fibre optic tip = 4.25 mW) and with an amber LED (595 +/- 20nm, Doric, maximum power at fibre optic tip = 5 mW). LED module output was controlled using the Igor Pro 6.37 software. Laser photo-inhibition experiments were also performed with a green laser (MatchBox series, 532 +/- 0.5 nm, maximum power at fibre optic that was used approx. 40 mW). In these experiments the data were acquired at a rate of 10 kHz using Igor Pro 6.37. The laser was operated manually, and the light duration was recorded using an Arduino Uno board that created a digital time stamp. Experiments were only included if the laser illumination duration was between 19.6 - 20.7 seconds. The area of light illumination was estimated to have a diameter of 1 - 2 mm and therefore for a power of 10 mW the light intensity was between 0.8 - 3.2 mW / mm².

**Histology and imaging**

After electrophysiological recordings, acute brain slices were fixed in 4 % PFA overnight. Slices were kept in PBS (Phosphate Buffered Saline: 1.37 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄) at 4 °C for short-term storage. For biocytin labelling the slices were washed with 1X PBS 3-4 times and permeabilized with freshly prepared 0.3 %-Triton 1X PBS for 4 - 5 hours. Streptavidin conjugated to Alexa FluorTM 488 (Invitrogen S32355) in PBS-Triton 0.3 % (1:500) was incubated overnight at 4 °C. The slices were then washed 4 - 5 times in PBS for 2 hours. Slices were mounted on glass slides using mounting media (DAKO). Confocal images (1024 x 1024) were acquired on a Zeiss LSM700 upright confocal microscope using the 10x air objective and digitally captured using the default LSM acquisition software. Pyramidal cell reconstruction was performed on neuron studio and simple neurite tracer plugin on Fiji. Fluorescence expression was quantified using 40 pixel wide line profiles through the layers of CA3 in Fiji, with background subtraction, and the signal normalised to the background.
Analysis of local field potentials

In order to characterise and analyse the oscillations, a Hanning window was applied and the power spectra were calculated as the normalised magnitude square of the FFT (Igor Pro 6.37). The 50 Hz and 100 Hz frequencies were not included in the analysis to exclude the mains noise and its harmonic component. The oscillation amplitude was quantified firstly by measuring the peak of the power spectrum termed as peak power and secondly by measuring the area below the power spectrum plot in the gamma-band range (20 - 100 Hz) termed as power-area. The peak frequency of the oscillation was obtained by measuring the frequency at which the peak of the power spectrum occurred in the gamma-band range. In order to quantify when Cch-induced oscillations where abolished upon light stimulation, and to exclude the peak frequencies of those oscillations from further analysis, one of the two criteria had to be met. Firstly, an auto-correlation of the oscillations was computed and was fitted with a Gabor function \( f(x) = (A \times \cos(2\pi \times f \times x)) \times e(-x^2/2 * \tau) \). The first criterion was met if the resulting Gabor fit had a linear correlation coefficient, \( r > 0.7 \) and \( \sqrt{f \times \tau} > 0.1 \) (> 0.15 for frequencies higher than 50 Hz). The second criterion was a power-area larger than 125 \( \mu V^2 \) in the range of +/- 5 Hz of the peak frequency. The power-area was always included in the analysis even if oscillations were abolished. The power spectrum analysis for de novo oscillations was performed in the range of 52 - 149 Hz with the only criterion for oscillation presence being that the power-area in +/- 5 Hz of the peak frequency was larger than 40 \( \mu V^2 \). Hilbert transforms were used to obtain instantaneous gamma magnitude for sinusoidal modulation of gamma oscillations (band-pass filtered 20 - 120 Hz). For visualisation purposes the magnitude of the continuous wavelet transform was used normalised by max value (Morlet wavelet; \( \omega = 6 \)).

Spike detection and analysis
Unit detection was performed using custom-written procedures in MATLAB (2015-17, Mathworks).

Extracellular spikes from the 16 channel MEA were detected as described before by Quiroga and colleagues (Quiroga, Nadasdy & Ben-Shaul, 2004; Quian Quiroga, 2009). Briefly, the MEA data were processed with an elliptical band-pass filter (for spike detection: 4th order, 300 - 3000 Hz, for spike sorting: 2nd order, 300 - 6000 Hz). Spikes were detected as signals exceeding 5 standard deviation (s.d.) of the noise, $5 \times \sigma_n$, where $\sigma_n = \text{median} \{|x| / 0.6745\}$. Signals that exceeded 10 times the s.d. of the detected spike amplitudes were eliminated as artefacts/population spikes. Subsequently, spikes that had peaks occurring at the same time (< 0.1 ms) across channels were grouped together as one unit. This prevented detection of the same unit more than once. Clustering of the detected spikes was performed using custom-written procedures in Igor Pro 6.37. A spike sorting procedure adapted from Fee and colleagues was used to explore whether neurons displaying specific spike waveforms were selectively recruited by optogenetic stimulation (Fee, Mitra & Kleinfeld, 1996).

Briefly, spike metrics were converted into z scores, over-clustered using an in-built k-means algorithm, and progressively aggregated if the intercluster distance was <2.5 and merging did not produce more violations of refractory period of 2 ms. Analysis was performed on the clustered spikes, with auto-correlation and cross-correlation plots used to validate the clustering procedure. Spike metrics from the average waveform for each cluster were used to identify different waveform types via a k-means algorithm. This clustering procedure is likely to be conservative, and underestimate the firing rate of individual neurons, but was deemed sufficiently robust to detect any bias in optogenetic recruitment. A single unit cluster was identified if it 1) had less than 1.4 % of its total spike waveforms within 2 ms of its refractory period and 2) consisted of more than 800 members. When a cluster did not obey these criteria, it was merged with other clusters that had similar action potential waveforms giving rise to a multi-unit cluster.

Clusters were identified as expressing ChR2 if the spike rate in the first 100 ms of the step stimulus was 3 s.d. above the baseline spike rate. The remaining clusters were classified based on the delay.
between the negative and positive peaks in the average waveform as fast-spiking (<0.6 ms) or regular-spiking (>=0.6 ms). The Activation Index was calculated over the last second of the step stimulus as the difference between the light-induced and baseline spikes rates divided by their sum, and designed to measure sustained firing. The Theta Modulation Index was calculated as the rank correlation coefficient between the spike time histogram and the theta-modulated amplitude of the light stimulus.

**Computational modelling**

The activity in populations of excitatory and PV+ inhibitory neurons were modelled using Wilson-Cowan firing rate equations (Devalle, Roxin & Montbrió, 2017):

\[
\tau_m \dot{R} = -\dot{R} + G(D_{syn, total} + D_{ext})
\]  

(1)

where \(\tau_m\) is the membrane time constant, \(R\) is the mean firing rate (and the overdot indicates a derivative taken with respect to time), \(D_{syn, total}\) is the total synaptic drive, \(D_{ext}\) is the external drive to mimic carbachol induced depolarisation or optogenetic manipulation, and \(G(x)\) is the population response function:

\[
G(x) = \frac{1}{\sqrt{2\pi\tau_m}} \cdot \sqrt{x + \sqrt{x^2 + \Delta^2}}
\]  

(2)

This includes the parameter \(\Delta\) to represent heterogeneity, which was set at 0.3 throughout.

The activity in a population of SST+ interneurons was modelled using macroscopic equations derived from quadratic integrate-and-fire neurons (Devalle, Roxin & Montbrió, 2017):

\[
\tau_m \dot{R} = \frac{\Delta}{\pi \tau_m} + 2RV
\]  

(3)
\[ \tau_m \dot{V} = V^2 - (\pi \tau_m R)^2 + D_{\text{syn, total}} + D_{\text{ext}} \]  

(4)

which include the influence of mean membrane voltage \( (V) \), and enable oscillations in a lumped model of inhibitory neurons. \( \tau_m \) was 10 ms for excitatory cells, and 5 ms for both populations of inhibitory cell.

For all three neuronal populations, the total synaptic drive was the sum of excitatory and inhibitory inputs each cell population received, with each modelled as exponentially decaying synapses:

\[ D_{\text{syn, total}} = \sum_i D_{\text{syn}_i} \]  

(5)

\[ D_{\text{syn}_i} = w_i \tau_m S_i \]  

(6)

\[ \tau_{\text{syn}} \dot{S}_i = -S_i + R_{\text{pre}_i} \]  

(7)

where \( w \) is the synaptic weight (Table 1), \( S \) is the variable for synaptic activation, \( \tau_{\text{syn}} \) is the synaptic time constant (Table 1) and \( R_{\text{pre}_i} \) is the respective presynaptic spike rate. The relative synaptic weights were based on synaptic currents recorded in excitatory and inhibitory neurons during cholinergically-induced gamma oscillations (Oren et al., 2006). The synaptic time constants were tuned to give \( \sim35 \) Hz oscillations across a range of \( I_{\text{ext}} \) in excitatory cells (6-12).

The differential equations were solved in MATLAB 2019a using the ode23tb function and a 0.1 ms time step. The signal used to analyse rhythmic activity was the inhibition in the E cells, calculated as the sum of \( wS \) for the input from PV+ and SST+ neurons, as cholinergically-induced LFP oscillations in the hippocampus ex vivo appear to reflect inhibitory currents in pyramidal neurons (Mann et al., 2005; Oren, Hájos & Paulsen, 2010). Similar to the analysis of the LFP, the frequency was only
reported if, i) the Gabor fit to the auto-correlation showed $r > 0.7$ and $\sqrt{f \cdot \tau} > 0.1$, and ii) the peak power in the gamma range was $>1$. The peak power was calculated for all simulations.

**Statistics**

Repeated measures ANOVA (rmANOVA) was performed in SPSS 24 with a Greenhouse-Geisser correction where required (i.e. significance in Mauchly’s test for sphericity) and followed by Bonferroni-corrected post-hoc paired t-tests. Linear correlations, circular correlations, and Bonferroni-corrected one sample t-tests were performed using Igor Pro 6.37. Spearman’s Rank correlations were performed in SPSS 24. Scatter-bar charts were generated using PRISM 7. Circular statistics of spike phase relative to ongoing oscillations in the LFP were calculated using in-built functions in Igor Pro 6.37. The measurements spiking rates deviated from normality, and were analysed using non-parametric statistical tests performed in SPSS 24: differences between cell types were analysed using Kruskal-Wallis Test, followed by posthoc Dunn’s test, with Bonferroni correction for multiple comparisons. Differences across stimulus types (step and theta) were analysed using the Wilcoxon signed rank test, and the significance of modulation indices analysed using the one-sample Wilcoxon signed rank test ($H_0=0$). Error bars in graphs represent the standard error of the mean, unless explicitly stated otherwise. Stars represent significance values where * $p <0.05$, ** $p <0.01$ and *** $p <0.001$.

**Results**

**PV+ interneuron activity is necessary for cholinergically-induced gamma oscillations in hippocampal CA3**

In order to test if the activity of PV+ interneurons is necessary for the generation of slow hippocampal gamma oscillations, we took advantage of optogenetic photo-inhibition (Chow et al.,
We injected PV-cre mice with AAV carrying the inhibitory proton pump archaerhodopsin (Arch3-eYFP or ArchT-GFP). Expression of Arch in PV-cre mice was restricted to the pyramidal cell layer indicating preferential expression in perisomatic targeting PV+ interneurons (Fig. 1A & 2B) (Somogyi & Klausberger, 2005; Royer et al., 2012; Hu, Gan & Jonas, 2014). Intracellular recordings performed in opsin expressing cells demonstrated that these cells were fast-spiking and that sustained light illumination was able to produce robust hyperpolarisation, indicating functional expression of Arch in PV+ interneurons (Fig. 1B&C).

Gamma oscillations were induced in hippocampal slices from PV-Arch mice in area CA3 using bath application of the cholinergic agonist carbachol (Cch - 5 μM). Local field potential recordings from the CA3 pyramidal cell layer revealed robust gamma oscillations that were centred around 30 – 40 Hz (Fig 1D-F), as has been reported previously (Fisahn et al., 1998; Hájos et al., 2004; Mann et al., 2005). In each optogenetic experiment the photo-excitation protocols were repeated multiple times (4 – 10 trials) and the mean response reported. Overall, sustained photo-inhibition of PV+ interneurons using LED illumination (< 5mW) significantly decreased gamma power area (0.82 +/- 0.068 of baseline period, t = 2.59, p = 0.029, one sample t-test; Fig. 1E&G), although increases in power were observed in some slices (Fig. 1G). A significant suppression was also observed in the period of 0.5 - 1.5 seconds following light illumination termination (0.85 +/- 0.022 of baseline period, t = 6.70, p < 0.001, one sample t-test; Fig. 1G). The light-induced changes in gamma power were reversible, as there were no significant changes in the gamma power area recorded during the baseline periods across trials (F(4, 116) = 0.68, p = 0.61, rmANOVA). In addition, the changes in gamma power were not accompanied by a consistent alteration in gamma frequency (F(1.44, 43.23) = 1.25, p = 0.288, rmANOVA; Fig. 1H), although there was a significant correlation between the changes in frequency and power area (t = 2.77, p = 0.01, Pearson correlation, Fig. 1I), suggesting a consistent modulation of endogenous oscillatory activity.
While LED photo-inhibition of PV+ interneurons significantly modulated gamma power, the oscillations did not collapse. Pyramidal neurons make strong recurrent connections with PV+ interneurons (Mann, Radcliffe & Paulsen, 2005; Oren et al., 2006; Hofer et al., 2011; Packer & Yuste, 2011; Bartos & Elgueta, 2012; Kohus et al., 2016), and it might be hard to break these feedback loops with photo-induced inhibitory currents. To test this possibility, we used long-lasting laser illumination with the prospect of biochemically silencing PV+ interneurons, by preventing synaptic release via terminal alkalisation (El-Gaby et al., 2016). PV+ interneurons expressing ArchT-GFP were illuminated with sustained green laser light (532 nm, approx. 18 mW for 20 seconds). Similar to the LED experiments, there were inconsistent network responses to PV+ interneuron photo-inhibition at the beginning of laser illumination (1.00 +/- 0.087 of baseline period, t = 0.04, p = 1.00, one sample t-test; Fig. 1J&K). However, the power of the oscillation consistently decreased during sustained laser illumination (0.57 +/- 0.086 of baseline period, t = 5.00, p < 0.001, one sample t-test; Fig. 1J&K) and remained suppressed in first 10 seconds following laser stimulation (Post1: 0.78 +/- 0.071 of baseline period, t = 3.17, p = 0.030, one sample t-test; Fig. 1J&K), but eventually recovered (Post2 : 0.98 +/- 0.04 of baseline period, t = 0.43, p = 1.00 , one sample t-test; Fig. 1J&K). There was no consistent effect on the frequency of the oscillations (F(1.93, 23.21) = 7.29, p = 0.004, rmANOVA; paired t-tests to baseline, t <2 .692, p > 0.078, Fig. 1J&L). Laser illumination of PV+ interneurons expressing only control fluorophore did not alter gamma oscillation power nor frequency (data not shown). This slow and selective process of decreasing gamma power is consistent with biochemical silencing of synaptic terminals (El-Gaby et al., 2016). These results further support the importance of PV+ interneuron activity in generating gamma oscillations in hippocampal area CA3 (Hájos et al., 2004; Mann et al., 2005; Gulyás et al., 2010; Tukker et al., 2013). Residual gamma oscillations following photo-inhibition of PV+ interneurons may reflect incomplete transfection of the PV+ network or the presence of a distinct oscillatory circuit.
SST+ interneurons are necessary for Cch-induced gamma oscillations in hippocampal area CA3

To examine if SST+ interneuron activity is also required during Cch-induced gamma oscillations in CA3, we injected the AAV-Arch vector (Arch3-eYFP or ArchT-GFP) intrahippocampally in SST-cre mice. Expression of Arch was restricted to the strata oriens, radiatum and lacunosum moleculare (Fig. 2A&B), suggesting expression in SST+ dendrite-targeting interneurons (Ma et al., 2006; Lovett-Barron et al., 2012; Muller & Remy, 2014; Urban-Ciecko & Barth, 2016). Whole-cell recordings were performed in opsins positive cells and indicated functional expression of Arch (n = 4, Fig. 2C&D). SST+ interneurons often showed a pronounced sag during current- and light-induced hyperpolarisation (Fig. 2C&D), and could show rebound spikes following light pulses and steps (data not shown), but remained hyperpolarised throughout photo-stimulation.

Unlike the experiments with PV+ photo-inhibition, sustained photo-inhibition of SST+ interneurons using LED illumination (< 5mW) reliably decreased gamma oscillation power (0.69 +/- 0.057 of baseline period, t = 5.40, p < 0.001, one sample t-test; Fig. 2E-G), which remained suppressed in the immediate period following SST+ interneuron photo-inhibition (0.76 +/- 0.039 of baseline period, t = -6.26745, p < 0.001, one sample t-test; Fig. 2G). This post-light suppression was reversed from trial to trial (F(4, 164) = 2046, p = 0.048, rmANOVA; all paired t-tests t > 2.81, p > 0.07). In addition, light stimulation significantly modulated oscillation frequency (F(1.25, 39.97) = 22.60, p < 0.001, rmANOVA), with an increase in frequency from 37.79 +/- 1.083 Hz to 43.00 +/- 1.466 Hz during light stimulation (t = 4.74, p < 0.001, paired t-test), which reversed following light offset (Fig. 2H). There was also a significant correlation between the changes in frequency and power area of the oscillations (t = 3.11, p = 0.004, Pearson correlation, Fig. 2I), suggesting again a consistent modulation of endogenous oscillatory activity.
In the first 10 s of stronger laser illumination (532 nm, approx. 18 mW, 20 s total duration) similar effects were observed as in the LED experiment. Specifically, the power of Cch gamma oscillations decreased (0.70 +/- 0.064 of baseline, t = 4.76, p < 0.001, one-sample t-test), and the peak frequency increased (34.22 +/- 1.191 Hz to 38.60 +/- 1.868 Hz, t = 3.93, p = 0.011, paired t-test; rmANOVA, F(1.79, 17.87) = 4.61, p = 0.028; Fig. 2J-L). During the second half of the stimulation period (10 - 20 s), gamma power was strongly suppressed (0.35 +/- 0.090 of baseline, t = 7.23, p < 0.001, one sample t-test; Fig. 2J-L), often resulting in oscillation collapse (7/13 slices). This could indicate that silencing SST+ interneurons is sufficient to disrupt the hippocampal network during gamma oscillations and that SST+ interneuron activity is necessary for proper maintenance of Cch-induced oscillations in the CA3 area of the hippocampus. Moreover, the robust upregulation of gamma oscillation peak frequency (Fig. 2J&L) suggests that SST+ interneurons can exert strong control over the frequency of slow gamma oscillations.

**Rhythmic synchronisation of the hippocampal network by perisomatic and dendritic inhibition**

The experiments using photo-inhibition indicate that the generation of gamma oscillations in hippocampal area CA3 involves the endogenous recruitment of both PV+ and SST+ interneurons. In order to test whether the activation of PV+ or SST+ interneurons is sufficient to entrain the hippocampal network at gamma frequencies, we next examined cell type-specific photo-excitation using Channelrhodopsin 2 (ChR2) (Nagel et al., 2003; Boyden et al., 2005). Injection of AAV-ChR2-mCherry produced similar expression patterns as Arch in both PV- and SST-Cre mouse lines (Fig. 3A&B). Photo-excitation of ChR2-expressing PV+ interneurons at 40 Hz (1-5 ms pulse width) reliably evoked spikes throughout pulse trains (median spike rate [interquartile range (IQR)] = 40.5 [40.3, 43.3] Hz; median spike fidelity [IQR] = 1.0 [1.0, 1.0]; n=5; Fig. 3C&D), and entrained ongoing
oscillations in 14/18 experiments (>2mW; n = 12 at 5.5 mW, n = 6 at 2.2 mW - merged due to similar effects; Fig. 3E,G&I). In the remaining 4 out of 18 experiments the ongoing oscillations were not entrained (Fig. 3I). This effect was likely observed due to low ChR2 expression, as pulses with longer width (5 ms) entrained the oscillation in the same experiments (Fig. 3J). Thus, PV+ interneurons are sufficient to synchronise the hippocampal network at gamma frequencies.

Rhythmic photo-excitation of SST+ interneurons evoked spikes throughout pulse trains (median spike rate [IQR] = 41.7 [40.4, 71.3] Hz; median spike fidelity [IQR] = 1.0 [1.0, 1.0]; n=13; Fig. 3C&D). This stimulation pattern reliably entrained ongoing oscillations in 19 out of 22 experiments (>2mW; n = 13 at 5.5 mW, n = 9 at 2.2 mW - merged due to similar effects; Fig. 3F,H&K). In the remaining 3 out of 22 experiments, oscillations were abolished during 40 Hz photo-excitation. These results indicate that transient activation of SST+ dendrite-targeting interneurons is also sufficient to synchronise the hippocampal network at gamma frequencies. Activation of PV+ and SST+ interneurons produced opposite deflections in the pulse-locked waveform of the LFP recorded in the stratum pyramidale (Fig. 3E-H), as might be expected from the somatodendritic profile of their axon terminations. However, activation of SST+ interneurons was sometimes accompanied by an initial fast negative component (Fig. 3H), which was reminiscent of a population spike arising from the synchronised firing of excitatory cells in the hippocampus (Andersen, Bliss & Skrede, 1971; Wierenga & Wadman, 2003), despite the sparsity of SST+ axons in this layer.

To study the SST+ induced waveform in isolation, we repeated the same experiment in quiescent slices, perfused only with aCSF. Blue light pulses (1 ms width) at 40 Hz induced strong pulse-locked field responses with fast-negative deflections, which were resistant to glutamate receptor blockers (Fig. 4A-C), but were followed by a glutamate receptor-mediated positive deflection. Application of GABA_A receptor (GABA_AR) blockers reduced the overall amplitude of the phase-locked field responses (n = 3; Fig 4D-E). Furthermore, application of GABA_AR blockers lead to light-induced
epileptiform bursts (n = 4; Fig. 4F) during 2 s sustained photo-excitation. These results suggest that SST+ interneuron photo-excitation generates network excitation, that is not mediated through GABA<sub>A</sub>Rs, at the onset of light illumination. We did not observe ChR2 expression in CA3 pyramidal neurons during whole cell recordings, but they did receive weak excitatory postsynaptic currents (EPSCs) throughout light illumination (Fig 4G). Light-evoked inhibitory postsynaptic currents current (IPSCs) were always larger than evoked EPSCs, and perforated patch-clamp recordings in current-clamp mode revealed that pyramidal cells were inhibited during SST+ interneuron photo-excitation (n = 18, Fig. 4G-I). Activation of GABA<sub>B</sub>R may contribute to the dominant effect of membrane hyperpolarisation, as these receptors were blocked by QX-314 during voltage-clamp recordings. However, this does suggest that there are some off-target effects in slices from SST-ChR2 mice.

Sustained activation of PV+ interneurons suppresses Cch-induced gamma oscillations

We used two patterns of sustained activation in slices from PV-ChR2 mice: light steps to drive tonic firing and fully-modulated sine waves at 8 Hz to mimic excitatory input during theta-frequency oscillations (Buzsáki, 2002). In a subset of light step experiments, we recorded ongoing gamma oscillations in the LFP whilst tonically driving PV+ interneurons at increasing strengths across trials (by changing the levels of blue light illumination, 10 - 5500 μW). The change in power between baseline and light activation period was measured at each light intensity level. We then obtained the response level at which the power changed by half of the maximum for each experiment (half-maximal response). For half-maximal response trials, photo-excitation of PV+ interneurons (2 seconds) consistently decreased the power-area (0.52 +/- 0.016 compared to baseline, t = -29.56, p < 0.001, one-sample t-test; Fig. 5A-C & E) and increased the peak frequency (from 32.70 +/- 0.793
Hz (baseline) to 38.76 +/- 1.094 Hz, t = 8.21, p < 0.001, paired t-test; Fig. 5D&F). Furthermore, there was a progressive decrease in power ($r = -0.84$, $n = 121$ values, $t = 17.00$, $p < 0.001$; Fig. 5G) and increase in frequency ($r = 0.49$, $n = 100/121$ values, $t = 5.60$, $p < 0.001$; Fig. 5H) as the light intensity increased. In order to estimate the maximal effect of PV+ interneuron stimulation, we pooled experiments using strong light intensity illumination (> 2mW, including cases where light intensity-response curves were not assessed; $n = 14$ at 5.5 mW, $n = 9$ at 2.2 mW). Overall, strong light illumination caused a substantial decrease in the normalised power-area (0.09 +/- 0.029, $t = 31.07 p < 0.001$, one-sample t-test; Fig. 5I&J) and abolished the oscillations in most experiments (17/23).

These results indicate that progressive up-regulation of PV+ interneuron activity decreases gamma power and increases the frequency until the rest of the hippocampal network is fully silenced.

Interneurons have been shown to be particularly susceptible to depolarisation block (Herman et al., 2014), and we did observe this in one of the current-clamp recordings from PV+ interneurons during step illumination (median spike rate [IQR] = 30.6 [8.4, 91.1] Hz; $n = 6$; excluding 1 neuron showing depolarisation block). This seems unlikely to explain the effects we observe, as photo-stimulation in aCSF did not induce increases in network activity (Fig. 5K&L). In order to examine directly whether PV+ interneurons could maintain spiking with sustained photo-excitation during Cch-induced oscillations, we recorded spiking activity using a linear multi-electrode array (MEA) (Fig. 6A). PV+ interneurons (spike width: 0.49 +/- 0.04 ms) showed variability in spike rates (median spike rate [IQR] = 44.5 [17.3, 108.8] Hz; $n = 18$), but this activity was maintained during sustained illumination (5.5 mW; Median sustained activation index [IQR] = 0.87 [0.46, 1], $Z=171$, $p<0.001$, $n=18$, one-sample Wilcoxon signed rank test; analysis performed on last second of trial), and was associated with decreased activity of regular spiking ([RS; -0.72 [-0.92, -0.40]; $Z=2$, $p<0.001$, $n=53$, one-sample Wilcoxon signed rank test; $z=65.7$, $p<0.001$ cf. PV+ interneurons, Kruskal-Wallis Test followed by posthoc Dunn’s test with Bonferroni correction for multiple comparisons) and fast-spiking cells (FS; -0.52 [-0.82, -0.12]; $Z=141$ $p<0.001$, $n=49$, one-sample Wilcoxon signed rank test; $z=50.1$, $p<0.001$
cf. PV+ interneurons, Kruskal-Wallis Test followed by posthoc Dunn’s test with Bonferroni correction for multiple comparisons) (Fig. 6B-D&G). Current-clamp recordings from putative pyramidal neurons confirmed that light stimulation produced sustained membrane hyperpolarisation (Fig. 6I).

These results are consistent with increased PV+ interneuron activity during light illumination that leads to reduced activity in hippocampal principal cells.

During 8 Hz sinusoidal modulation of PV+ interneurons, the instantaneous gamma magnitude, assessed using the Hilbert transform, was found to be negatively correlated with light intensity in agreement to light step experiments (across all experiments, Pearson correlation, mean $r = -0.51 +/ - 0.04$, $t > 23.2$, $p < 0.001$, $n = 12$) (Fig. 6H). During MEA recordings, spike rates of PV+ interneurons correlated positively with theta-frequency changes in light intensity (Median rank correlation coefficient [IQR] = 0.75 [0.55, 0.83], $Z=120$, $p=0.001$, $n=15$, one-sample Wilcoxon signed rank test), while negative correlations were found for the spike rates of RS ($-0.19 [-0.37, -0.09]$; $Z=100$, $p<0.001$, $n=43$, one-sample Wilcoxon signed rank test; $z=48.3$, $p<0.001$ cf. PV+ interneurons, Kruskal-Wallis Test followed by posthoc Dunn’s test with Bonferroni correction for multiple comparisons) and FS cells ($-0.19 [-0.39, -0.01]$; $Z=232$, $p=0.006$, $n=42$, one-sample Wilcoxon signed rank test; $z=45.7$, $p<0.001$ cf. PV+ interneurons, Kruskal-Wallis Test followed by posthoc Dunn’s test with Bonferroni correction for multiple comparisons) (Fig. 6E). All PV+ interneurons recorded during ongoing slow gamma oscillations showed significant phase-coupling ($p<0.05$, Rayleigh test), with a mean spike phase of $-1.8 [-2.3, -0.9]$ radians (second-order mean [95% confidence intervals]; $n=10$; Fig. 7F&G).

The PV+ interneurons fired at a significantly later phase of the oscillation than the RS cells ($F(2,55) = 5.36$, $p = 0.007$, Two-sample Hotelling test). These findings indicate that PV+ interneurons are synchronised within slow gamma, and that a transient increase in PV+ interneuron activity causes a rapid and reversible decrease in the power of the Cch-gamma oscillations and firing rates of other neurons.
Sustained activation of SST+ interneurons induces fast gamma oscillations

We obtained the light intensity response curves with light steps in slices from SST-ChR2 mice and observed similar results as in PV-ChR2 experiments. Sustained light illumination decreased the power (0.49 +/- 0.029, t = 17.53, p < 0.001, one sample t-test; Fig. 7A-C & E) and increased the frequency at half-maximal response (from 34.08 +/- 0.954 Hz during baseline period to 38.17 +/- 1.400 Hz, t = 3.658, p < 0.01, paired t-test; Fig. 7D&F). Moreover, as the light intensity increased, the power progressively decreased (r = -0.66, n = 107 values, t = 9.11, p < 0.001; Fig. 7G), and frequency progressively increased (r = 0.71, n = 56 out of 107 values, t = 7.41, p < 0.001; Fig. 7H). It is perhaps not surprising that excitatory networks can be suppressed by photo-excitation of GABAergic interneurons. However, different responses were revealed when we assessed the effects of strong photo-excitation of SST+ interneurons on Cch-induced gamma oscillations (light-intensity response curves where performed in a subset of experiments; n = 18 slices at 5.5 mW and n = 13 slices at 2.2 mW, merged). Similar to PV-ChR2 step experiments, the gamma power was reduced during light stimulation when compared to baseline period (0.34 +/- 0.150, t = -4.39, p < 0.001, one sample t-test; Fig. 7I&J) and in approximately half of the experiments, gamma oscillations were fully abolished (n = 16/31 slices). In contrast, in experiments where the oscillations persisted, their frequency increased strongly from 34.63 +/- 0.836 Hz during baseline to 62.75 +/- 4.921 Hz during light illumination (n = 15/31 slices; t =5.61, p < 0.001, paired t-test; Fig 7I-K). These fast gamma oscillations occurred most reliably in slices for which the light-intensity response curves were not obtained. In order to test if SST+ interneuron photo-excitation alone is sufficient to induce oscillations, as opposed to simply increasing the frequency of ongoing activity, we repeated the same experiments in the absence of Cch. Sustained photo-excitation of SST+ interneurons induced de novo oscillations.
in the fast gamma-band range with peak frequency of 80.5 +/- 2.48 Hz (12/16 slices; Fig. 8A,C&D).

Isolating the CA3 area from DG did not prevent the generation of *de novo* oscillations (n = 3 slices).

Furthermore, sinusoidal light activation at 8 Hz (theta photo-excitation) also induced robust oscillations with higher peak frequency than the tonic activation 111.2 +/- 3.15 Hz (13/17 slices; t = 7.64, p < 0.001, two-sample t-test; Fig. 8B-D). This is consistent with previous experiments showing that transient light activation induces higher frequency oscillations than sustained illumination (Butler *et al*., 2016; Betterton *et al*., 2017). Furthermore, the power (r = 0.67, n = 70 values, t = 7.52 p < 0.001) and frequency (r = 0.77, n = 48/70 values, t = 8.20, p < 0.001) of the *de novo* oscillations progressively increased as the light intensity of theta photo-excitation was elevated (Fig. 8E&F). This monotonic increase in peak frequency contrasts with the properties of oscillations induced by photo-excitation of principal cells in the hippocampus, where the frequency of the oscillations remains relatively constant within the slow gamma band across light intensities (Butler *et al*., 2016; Betterton *et al*., 2017; Butler, Hay & Paulsen, 2018). Therefore, SST+ interneuron photo-excitation in CA3 appears to induce a distinct type of gamma activity.

The fast gamma oscillations that emerge during sustained photo-excitation of SST+ interneurons could reflect the intrinsic synchronisation of SST+ networks, but there are a number of possible scenarios in which this stimulation paradigm could lead to the activation of other hippocampal microcircuits involving network excitation. Depolarising GABA could contribute to recruitment of postsynaptic targets, but perforated patch recordings from hippocampal cells in stratum pyramidale (aCSF only) showed that they were hyperpolarized by light illumination (Fig. 4H&I). Alternatively, network excitation and oscillogenesis could emerge following depolarisation block of SST+ interneurons, and subsequent disinhibition, but direct photo-inhibition of SST+ interneurons was not able to generate *de novo* oscillations (Fig. 7L&M). However, the power of the light-induced oscillations was markedly reduced following block of either fast excitation or inhibition (Fig. 8G&H).
This suggests that the light-induced oscillations recorded in LFP do not emerge solely from the GABAergic activity of SST+ interneurons.

Current-clamp recordings from SST+ interneurons during step illumination revealed that depolarisation block was quite common in these cells that were located close to the surface (median spike rate [IQR] = 43.8 [33.4, 75.7] Hz; n = 8; excluding 7 showing depolarisation block). To directly test if photo-excitation of SST+ interneurons leads to a dominant effect of depolarisation block during ongoing gamma oscillations, and whether photo-excitation is associated with net increases or decreases in the spiking activity of other neurons in the network, we performed MEA recordings (Fig. 8I-M). We found that SST+ interneurons (spike width: 0.69 +/- 0.03 ms) displayed robust activation (median spike rate [IQR] = 49.2 [29.5, 79.8] Hz; n = 68) that was sustained throughout the course of step stimulation (Median sustained activation index [IQR] = 0.90 [0.76, 0.98], Z=2346, p<0.001, n=68, one-sample Wilcoxon signed rank test; Fig. 8I,J&M), and faithfully followed the 8 Hz sine stimulation (Median rank correlation coefficient [IQR] = 0.63 [0.52, 0.72], Z=1484, P<0.001, n=54, one-sample Wilcoxon signed rank test; Fig. 8K&M). All but 3 of the SST+ interneurons recorded were significantly phase-coupled to the induced fast gamma-frequency oscillations (p<0.05, Rayleigh test), with a mean spike phase of -2.0 [-2.1, -1.8] radians (second-order mean [95% confidence intervals]; n=65; Fig. 8L,M). The RS and FS cells showed significantly weaker modulation (Fig. 8J,K), but did not appear to be suppressed as in the PV-ChR2 experiments, and rather showed an insignificant trends towards both increased activity during step illumination (Median sustained activation index [IQR]; RS: 0.12 [-0.08, 0.54], Z=50, p=0.13, n=11; FS: 0.49 [-0.14, 0.59], Z=24, p=0.09, n=7; one-sample Wilcoxon signed rank tests) and positive correlations with theta-frequency changes in light intensity (Median rank correlation coefficient [IQR]; RS: 0.26 [-0.07, 0.53], Z=49, p=0.16, n=11; FS: 0.28 [0.09, 0.57] Z=25, p=0.06, n=7, one-sample Wilcoxon signed rank tests). The majority of RS (8/11) and FS cells (4/7) were also significantly phase-coupled to the light-induced fast gamma oscillations (p<0.05, Rayleigh test), but did not show a consistent mean firing phase (RS:
Overall, this suggests that the dominant change in the network during the induction of fast gamma oscillations is a robust increase in the spiking of SST+ interneurons.

To explore whether the recruitment of SST+ interneurons might differ between step and theta stimulation, we analysed the maximum spike rates in the second half of the stimulation trials (20 ms bins). The maximum spike rates during theta stimulation were significantly higher than during the step stimulation ($Z=148$, $p<0.001$, $n=54$; Wilcoxon signed rank test). As theta stimulation induced faster gamma oscillations than step stimulation (see Fig. 8D), this further suggests that the frequency of fast gamma oscillations depends on the overall levels of SST+ interneuron excitation.

**Computational model of hippocampal gamma oscillations including PV+ and SST+ interneurons**

In order to provide mechanistic insight into how optogenetic manipulation of PV+ and SST+ interneurons impact hippocampal network dynamics, we developed a mean firing rate model including these two interneuronal subtypes. It has previously been shown that gamma oscillations in hippocampal CA3 can be modelled using Wilson-Cowan equations in an excitatory-inhibitory feedback loop (Akam et al., 2012). We therefore used these equations to model the excitatory pyramidal cell (E) and inhibitory PV+ interneuron populations. The activity in the population of SST+ interneurons was represented using equations derived from quadratic integrate-and-fire neurons (Devalle, Roxin & Montbrió, 2017), which enables oscillations within a mean firing rate model of an inhibitory network. Both interneuronal subtypes were reciprocally connected with E cells, but not with each other (Fig. 9A), with stronger excitatory connections to PV+ interneurons (Oren et al., 2006), and faster synaptic time constants in the E-PV+ loop (see Table 1). Moderate excitatory drive...
to the E population \( (D_{\text{ext}} = 6\text{-}12; \text{increments of } 1) \) was sufficient to induce gamma-frequency network oscillations (30\text{-}35 Hz; Fig. 9B), with little change in frequency with increasing drive (see Fig. 9C, for \( \Delta PV^+ \text{ drive } = 0 \text{ or } \Delta SST^+ \text{ drive } = 0 \)), consistent with the effects optogenetic activation of CA3 pyramidal neurons ex vivo (Butler, Hay & Paulsen, 2018). As expected from the synaptic connectivity and time constants implemented in the network, peak activity occurred first in the E cells, followed by the PV+ cells (3.7\text{-}3.9 ms) and then SST+ cells (4.4\text{-}9.9 ms; decreased monotonically with increasing E drive). This is consistent with the spike delays observed for perisomatic- and dendritic-targeting interneurons recorded during Cch-induced gamma-frequency oscillations ex vivo (Hájos et al., 2004). Oscillations were also observed at higher levels of drive to the E cells \( (D_{\text{ext}} > 12) \), but the peak in SST+ activity began to precede the peak in PV+ activity, due to the voltage-dependent acceleration of SST+ activation. The effects of manipulating interneuronal drive were thus examined across the range of conditions that appeared to best approximate the activity in ex vivo slices (E cells: \( D_{\text{ext}} = 6\text{-}12) \).

To mimic the effects of optogenetic manipulation using LEDs, a select interneuronal population received step changes in external drive (2 s), which varied from -10 to +10 across trials, with increments of 0.2. Moderate inhibition of the PV+ cells \( (PV \text{ cells: } D_{\text{ext}} = -0.2 \text{ to } -5.4) \) was ineffective at silencing PV+ cells and abolishing oscillations (Fig. 9B-D), due to corresponding increases in recurrent excitation. However, the increases in the firing rates of E and SST+ did have variable effects on network dynamics. Under some conditions, the increased activity in the slower E-SST+ loop reduced the frequency of network oscillations (Fig 9C). However, the delay between E and SST+ activity decreased with increasing activation (Fig. 9E), which tended to increase oscillation frequency, and so increases or decreases in oscillation frequency were observed depending on the initial conditions and degree of PV+ photo-inhibition (Fig 9C&D). Progressively stronger inhibition of PV+ cells was capable of at first disrupting rhythmicity, and eventually allowing sufficient disinhibition of E cells to trigger fast rhythmic activity in the SST+ cells (Fig. 9C&D). In contrast,
excitation of PV+ cells readily silenced E cells, and thereby abolished oscillatory activity (Fig. 9B-D).

These manipulations of PV+ cells had variable effects on oscillation frequency, but there was a significant negative correlation between peak frequency and peak power for both inhibition (Spearman’s rho = -0.48, n = 288, p < 0.001) and excitation (Spearman’s rho = -0.55, n = 66, p < 0.001).

To examine the effects biochemically silencing presynaptic PV+ terminals, we included a light-induced reduction in the strength of PV+ connections to E cells and themselves, with a maximum reduction of 50%, an onset time constant of 18.4 s, and recovery time constant of 13.1 s (El-Gaby et al., 2016). When combined with presynaptic silencing, a moderate inhibitory drive to PV+ cells ($D_{\text{ext}} = -5$) was sufficient to gradually reduce the amplitude of gamma-frequency oscillations over time, and eventually abolish rhythmic activity (Fig. 9F).

Applying an inhibitory drive to SST+ cells effectively silenced this population, due to weaker feedback excitation, with a predominant effect of reducing the peak power of gamma-frequency oscillations (Fig. 9B&C). For low levels of inhibition of SST+ cells (SST+ cells: $D_{\text{ext}} = -0.2$ to -2) there were variable effects on oscillation frequency, but stronger inhibition either produced a small but consistent increase in frequency (3.0 [2.2, 3.2] Hz; E cells: $D_{\text{ext}} = 6$ to 11) or abolished the oscillation (E cells: $D_{\text{ext}} = 11$ to 12), depending on the initial conditions (Fig. 9C&D). An increase in frequency of persistent oscillations was expected, as the slower synaptic time constants in the loop between E and SST+ cells tends to lengthen the cycle period, and there was a significant negative correlation between peak frequency and peak power (Spearman’s rho = -0.28, n = 274, p < 0.001). Combining inhibitory drive with presynaptic silencing gradually abolished the oscillation (Fig. 9F).

Excitation of SST+ cells also reduced the peak power of gamma-frequency oscillations (Fig. 9C), with corresponding changes in frequency best characterised into 3 phases with increasing drive: 1) the delay in the peak activity of E and SST+ cells decreased (Fig. 9E), increasing oscillation frequency, 2)
the increased activity of SST+ cells silenced E cells, abolishing oscillatory activity, and 3) the activity in SST+ cells reached sufficient levels to support intrinsic fast gamma-frequency oscillations (Fig. 9C&D). Under some conditions there were intermediate phases, such as a drop in frequency due to doublet spikes in SST+ activity on each cycle (E cells: $D_{ext} = 11$ to 12; SST+ cells: $D_{ext} = 2.8$ to 3.4). Overall, there was a significant negative correlation between peak power and oscillation frequency (Spearman’s rho = -0.33, n = 326, p < 0.001). This computational model of hippocampal oscillations indicates that SST+ interneuron sustained excitation is sufficient to generate gamma oscillations under certain conditions.

**Discussion**

Gamma oscillations depend on synchronised synaptic inhibition, and there is a wealth of evidence suggesting that perisomatic-targeting PV+ interneurons are critical for both current and rhythm generation (Mann et al., 2005; Bartos, Vida & Jonas, 2007; Oren, Hájos & Paulsen, 2010; Tukker et al., 2013; Cardin, 2016; Sohal, 2016; Penttonen et al., 1998). Here, we used optogenetic manipulation of PV+ and SST+ interneurons to explore whether PV+ interneurons have a selective role in gamma rhythmogenesis in the hippocampal CA3 ex vivo. Our findings suggest that disrupting interneuronal activity, via either photo-inhibition or photo-excitation, generally leads to a decrease in the power and increase in the frequency of ongoing cholinergically-induced slow gamma oscillations. This suggests that both PV+ and SST+ interneurons play key roles in maintaining slow gamma oscillations, and the key differences were that (i) gamma oscillations were more readily disrupted by photo-inhibition of SST+ rather than PV+ interneurons, (ii) manipulation of SST+ interneurons modulated gamma frequency more robustly than that of PV+ interneurons, and (iii) photo-excitation of SST+ interneurons could also induce *de novo* fast gamma oscillations. These key
differences were replicated in a mean firing rate model, in which excitatory neurons and PV+ interneurons were connected in a strong and fast feedback loop, and modelled using Wilson-Cowan equations, and SST+ neurons were modelled using equations derived from quadratic integrate-and-fire neurons that support interneuronal network oscillations.

Slow gamma oscillations in the hippocampal CA3 appear to be generated by synaptic feedback loops between excitatory pyramidal neurons and perisomatic-targeting interneurons, both in brain slices (Fisahn et al., 1998; Hájos et al., 2004; Mann et al., 2005; Oren et al., 2006; Butler, Hay & Paulsen, 2018) and in vivo (Bragin et al., 1995; Csicsvari et al., 2003; Fuchs et al., 2007). In such feedback loops, the period of the oscillation largely reflects the effective time course of inhibitory postsynaptic potentials in the pyramidal cells, which should become shorter with smaller compound inhibitory synaptic currents and/or increased pyramidal cell excitability. The amplitude of the oscillation recorded in the LFP also reflects the amplitude of phasic inhibitory currents in pyramidal neurons (Mann et al., 2005; Oren, Hájos & Paulsen, 2010), and during spontaneous gamma oscillations there is a strong correlation between the instantaneous period and amplitude of each gamma cycle (Atallah & Scanziani, 2009). One might thus expect disinhibition to decrease the amplitude and increase the frequency of gamma oscillations, which is largely what we observed with photo-inhibition of either PV+ or SST+ interneurons.

While photo-inhibition of SST+ interneurons was able to reliably disrupt gamma oscillations, it was necessary to use high-powered laser illumination of PV+ interneurons to consistently reduce gamma power, and the oscillations were not abolished under our stimulation paradigms. This is not inconsistent with PV+ interneurons playing a key role in the synaptic feedback loops generating gamma oscillations in the hippocampal CA3, as such a microcircuit should resist disinhibition. Indeed, it appears that strong laser illumination was necessary to biochemically silence PV+ interneuron terminals (El-Gaby et al., 2016), and thus break this feedback loop.
Our computational model supports these conclusions, and provides insight into a potential mechanism for the network effects of photo-inhibition. In this model, the E and PV+ cells are connected in a strong and fast feedback loop, which can oscillate alone at 60-70 Hz with external excitatory drive to the E cells (data not shown). The connections from E cells to SST+ cells are weaker and slower, and recurrent inhibition also has a slower time constant. Under baseline conditions, this loop slows the frequency of network oscillations to 30-35 Hz. However, as the SST+ population is modelled by macroscopic equations for quadratic integrate-and-fire neurons, membrane depolarization leads to sharp increases in firing rate, and can lead to the generation of fast gamma within the SST+ population. Photo-inhibition of SST+ cells reduces the influence of the slow E-SST+ loop, while increasing the firing rates of E and PV+ cells, leading to faster and weaker oscillations.

Photo-inhibition of PV+ cell firing rates is resisted by strong feedback excitation, but leads to increased activity of E cells, and greater synaptic excitation of SST+ cells. Depending on the initial conditions, this leads to increases or decreases in the oscillation frequency, which may explain the variable effects we observed ex vivo. It was possible to break the feedback loop by providing strong inhibitory drive to the PV+ cell population, which could unleash fast gamma in the SST+ cell population. We did not observe such effects ex vivo, which could reflect the power of the optogenetic inhibitors and/or light penetration. However, combining more moderate inhibition with presynaptic silencing was also effective in the model.

In ex vivo brain slices, we also found that photo-excitation of PV+ or SST+ interneurons led to an increase in frequency and decrease in power of gamma oscillations. This might be somewhat more surprising, but our model provides a relatively simple explanation. Photo-excitation of PV+ cells reduces E cell activity and the recruitment of SST+ cells, while photo-excitation of SST+ cells enables their accelerated recruitment due to membrane depolarization. Both of these effects reduce the slowing influence of E-SST+ loop observed during baseline conditions.
It was recently suggested that SST+ interneurons, but not PV+ interneurons, contribute to the generation of slow gamma oscillations in V1 (Chen et al., 2017; Veit et al., 2017; Hakim, Shamardani & Adesnik, 2018). Our results do not support an exclusive role for SST+ interneurons in slow hippocampal gamma oscillations, but are consistent with an important role for SST+ interneurons in gamma rhythmogenesis across cortical circuits. However, SST+ interneurons largely target the dendritic domains of pyramidal cells, and thus it remains difficult to see how they could directly contribute to the precise timing of pyramidal cell spiking during fast brain oscillations. SST+bistratified interneurons have similar properties to fast spiking PV+ interneurons, and also form a portion of synapses close to the soma (Somogyi & Klausberger, 2005; Muller & Remy, 2014), but have been reported to exhibit decreased GABA release under cholinergic stimulation (Gulyás et al., 2010). In our model, the dendritic location of SST+ is only represented by the slower time constant of inhibition, but it highlights a potentially important role of hippocampal SST+ interneurons in modulating the frequency of slow gamma oscillations that can be expressed in E-PV+ circuits.

While optogenetic manipulation of SST+ interneurons consistently disrupted slow gamma oscillations, we found that photo-excitation of SST+ interneurons could also induce de novo fast gamma oscillations. These GABAergic interneurons should provide a powerful source of circuit inhibition (Somogyi & Klausberger, 2005; Pfeffer et al., 2013; Taniguchi et al., 2011; Leão et al., 2012; Lovett-Barron et al., 2012, 2014; Royer et al., 2012; Urban-Ciecko & Barth, 2016), but we found that sustained photo-excitation of SST+ interneurons did not significantly inhibit the activity of ChR2-neurons, and that pulsed stimulation could drive network excitation. In our model, we could induce fast gamma via photo-excitation of SST+ cells, but this was accompanied by a strong suppression of activity in E and PV+ cells, and so it is likely that there are non-specific effects of ChR2 stimulation. There have been reports of off-target expression in juvenile SST-Cre mice (Taniguchi et al., 2011), so there is still a possibility for pyramidal cell expression that we could not detect, or even SST+ cells that co-release glutamate (Cattaneo et al., 2019). An alternative possibility is that robust activation...
of a dense plexus of SST+ axons in the dendritic layers is sufficient to induce spiking in pyramidal
neurons via ephaptic coupling (Anastassiou et al., 2011; Ferenczi et al., 2016) or changes in
extracellular ion concentration, which would be enhanced under interface recording conditions
(Octeau et al., 2019), and counteract the effects of synaptic inhibition. The generation of fast gamma
oscillations appeared to depend on the maintenance of network excitability, as the oscillations were
attenuated by block of iGluRs. However, the spiking of the majority of SST- RS neurons was only
weakly coupled to the phase of light-induced fast gamma oscillations, and without a consistent
population spike phase preference, while light-sensitive putative SST+ interneurons showed reliable
phase-locking. This could be consistent with fast gamma oscillations representing rhythmic
dendritic inhibition from SST+ interneurons, with only weak effects on the spike rate and timing of
other neurons in the network.

The mechanism by which a network of SST+ interneurons might generate fast gamma oscillations
remains obscure. In neocortex, SST+ interneurons avoid inhibiting each other (Pfeffer et al., 2013),
although there is evidence for sparse synaptic interactions between SST+ interneurons in the
hippocampus (Savanthrapadian et al., 2014), and for more generic coupling via gap junctions (Baude
et al., 2007). More experiments are required to resolve the mechanisms by which optogenetic
manipulation of interneurons influences hippocampal gamma oscillations, and whether SST+
neurons contribute to fast hippocampal gamma oscillations during theta and non-theta states in
vivo (Sullivan et al., 2011). However, our findings suggest that SST+ interneurons exert powerful
control over the power and frequency of slow hippocampal gamma oscillations, and contribute to
the generation of fast gamma states.
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**Figure legends**

**Figure 1:** Sustained photo-inhibition of PV+ interneurons suppresses the power of gamma oscillations. 

A, Confocal image of ventral hippocampus slice from a PV-cre mouse injected intrahippocampally with AAV-Arch3 eYFP. CA3 = Cornu Ammonis 3, DG = Dentate Gyrus, Pyr. = stratum pyramidale, Rad. = stratum radiatum, Or. = stratum oriens. Scale bar = 200 μm.  

B, Current clamp recording of an ArchT-GFP expressing PV+ cell from CA3 area, showing responses to depolarising and hyperpolarising current steps, and fast-spiking phenotype.  

C, Potent hyperpolarisation of four PV+ interneurons during green light illumination in aCSF (1.45 mW). 

D, Illustration of the electrophysiological setup, with coloured line indicating the region of CA3 stratum pyramidale from which recordings were obtained.  

E, Cholinergically-induced oscillations (5 μM Cch) were suppressed during PV+ interneuron photo-inhibition (LED, 530 nm, approx. 4.25 mW).  

F, Representative power spectra before (black) and during (green) LED illumination (arrows indicate peaks in the power spectra). 

G, Power area in the 20-100 Hz band normalised to baseline (Pre (Off)) during (On) and after LED stimulation (Off (Post)) (n = 35). 

H, Peak frequency for experiments when the oscillation was not abolished (n = 31/35). 

I, Change in power area plotted against change in peak frequency. 

J, Stronger photo-inhibition was achieved using high power laser illumination (approx. 18.6 mW). Top: Change in power area normalised to baseline and, Bottom: Peak frequency of the oscillation calculated in 1 second bins across experiments (n = 14).

K, Mean change in power area normalised to baseline (n = 14). 

L, Mean peak frequency for trials when the oscillation was not abolished (n = 13). *p < 0.05, ***p < 0.001, n.s. p >= 0.05. Changes in peak frequency were analysed using rmANOVA, followed by post-hoc paired t-tests with correction for multiple comparisons. Solid brackets represent paired t-tests and standalone star symbols represent one-sample t-test versus normalised baseline. Grey lines represent single experiments.
**Figure 2:** Sustained photo-inhibition of SST+ interneurons suppresses gamma power and increases frequency. **A,** Confocal image of ventral hippocampus slice from SST-cre mice with eYFP-Arch3 expression. Scale bar = 200 μm. **B,** Quantification of fluorescence expression profile in PV (n = 18) and SST (n = 21) ArchT-GFP expressing slices. **C,** Current clamp recording of an SST+ cell from CA3 area, showing responses to depolarising and hyperpolarising current steps. **D,** Potent hyperpolarisation of four SST+ interneurons during green light illumination (1.45 mW). **E,** Representative LFP recordings illustrating effect of SST+ interneuron photo-inhibition (LED, 530 nm, approx. 4.25 mW). **F,** Representative power spectra before (black) and during (green) LED illumination (arrows indicate peaks in the power spectra). **G,** Power area in the 20-100 Hz band normalised to baseline (Pre (Off)) during (On) and after LED stimulation (Post (Off)) (n = 44). **H,** Peak frequency for experiments when the oscillation was not abolished (n = 33/44). **I,** Change in power area plotted against change in peak frequency. **J,** Effects of sustained laser illumination (approx. 18.6 mW) on normalised power area (top) and peak frequency (bottom) calculated in 1 second bins (n = 17). **K,** Mean normalised power area (n = 17). **L,** Mean peak frequency for trials when the oscillation was not abolished (n = 11/17). *p < 0.05, **p < 0.01, ***p < 0.001, n.s. p >= 0.05. Solid brackets represent paired t-tests and standalone star symbols represent one-sample t-test versus normalised baseline. Grey lines represent single experiments.

**Figure 3:** Rhythmic photo-excitation of either PV+ or SST+ interneurons entrains Cch-induced gamma oscillations. **A-B,** Confocal images of ventral hippocampus (350 μm slice) from PV-cre (A) and SST-cre (B) mice with mCherry-ChR2 expression. Scale bar = 200 μm. **C-D,** Spiking responses of PV+ and SST+ interneurons during 40 Hz light pulses (1-5 ms pulse width), characterised in terms of spike rate (C) and spike fidelity (D). **E-F,** Entrainment of Cch-induced oscillations to 40 Hz light
pulses in PV-cre (E) and SST-cre (F) mice expressing mCherry-ChR2 (1 ms pulse width; blue light illumination at 5.5 mW), shown in LFP traces (top) and normalised wavelet spectrum (bottom).

Brighter colours represent larger magnitudes. **G-H**, Normalised average waveform following two consecutive 1 ms pulses at 40 Hz from each experiment (PV+: n = 15/18; SST+: n = 19/22). Bold line is the population average, thinner lines represent individual experiments and shaded area is the SEM. Arrows indicate initial negative peak. **I-K**, Peak frequency of oscillation before (Pre (Off)), during (On) and after (Post (Off)) light stimulation, for PV+ with 1 ms pulse width (I; n = 18/18), PV+ with 5 ms pulse width (J; n = 13/13; several overlapping traces) and SST+ with 1 ms pulse width (K; n = 19/22). Note that experiments entrained at 20 Hz reflect suppression of alternate gamma cycles.

**Figure 4: Network excitation arising from photo-excitation in SST-cre mice.** A, LFP responses to 40 Hz stimulation in slices from SST-ChR2 mice recorded in aCSF (1 ms pulse width; 5.5 mW). B, Average waveform before (black) and after application of 20 μM CNQX and 40 μM AP5 (orange). C, Effect of ionotropic glutamate receptor (iGluR) blockers on the amplitude of negative (t = -0.61, p = 0.58) and positive peaks (t = 3.49, p = 0.03, n = 4; paired t-test). iGluR blockers used: 20 μM CNQX, 40 μM AP5, n = 3; 20 μM CNQX, n = 1. D, Average waveform before (black) and after application of 10 μM gabazine (GBZ; orange). E, Effect of GBZ on absolute amplitude (n = 3). F, Photo-excitation of SST+ interneurons induces epileptiform bursts during following GABA\(\alpha\)R blockage (n = 2 at 20 μM bicuculine (BIC), n = 2 at 10 μM GBZ). G, (i) Voltage-clamp recording from putative pyramidal cell during photo-activation of SST+ interneurons (1.53 mW), and held at 0 mV (top) and -70mV (bottom) to isolate IPSCs and EPSCs, respectively. (ii) Across all cells, the mean EPSCs (blue) were smaller than IPSCs (orange) during SST+ interneuron photo-activation (n = 18). H-I, Perforated patch current-clamp recordings from putative CA3 pyramidal cell in SST-cre mice...
expressing ChR2-mcherry, showing responses to depolarising and hyperpolarising current steps 
(H), and hyperpolarisation in response to light stimulation (1.53 mW; n = 6). Grey traces represent 
individual cells, black trace the average and dark grey shaded area the SEM.

Figure 5: Sustained photo-excitation of PV+ interneurons decreases the power and increases the 
frequency of Cch-induced gamma oscillations. A-B, Representative LFP recordings from CA3 area 
illustrating effect of PV+ interneurons photo-excitation (155 μW) on gamma oscillations (A), along 
with its respective power spectrum (B; arrows indicate power spectrum peaks). C-D, Time course 
of normalised power area (C) and peak frequency (D), each calculated in 0.5 second bins across 
experiments (n = 12). E, Mean changes in normalised power area. F, Mean peak frequency; 
rmANOVA: F(1.14, 12.50) = 44.14, p < 0.001. G-H, Normalised power area (G) and changes in peak 
frequency (H) plotted against light intensity (n = 12). I, Strong and sustained blue light illumination 
(5.5 mW) induces a collapse of Cch-induced oscillations, as seen in LFP traces (top) and normalised 
wavelet spectrum (bottom). J, Effect of strong illumination on mean normalised power area (n 
total = 23; n = 14 at 5.5 mW and n = 9 at 2.2 mW). K-L, Strong and sustained blue light illumination 
does not induced increases in network activity in aCSF. ***p < 0.001, n.s. p >= 0.05. Solid brackets 
represent paired t-tests and standalone star symbols represent one-sample t-test versus 
normalised baseline. Grey lines represent single experiments.

Figure 6: Multi-unit recordings during PV+ interneuron sustained photo-excitation in 
hippocampal slices with Cch-induced gamma oscillations. A, Schematic diagram of the 
hippocampus illustrating MEA recordings during blue light illumination (5.5 mW) in CA3. B, 
Representative average spike waveforms of PV+ (single unit; green) and RS (multi-unit; black) 
eurons. C, Spike time histograms of the representative neurons during sustained light
illumination. **D-E**, Mean spike time histograms during step illumination (**D**) and sinusoidal theta
stimulation (8 Hz; **E**), calculated from the fraction of total spike counts in each bin for each
clustered neuron. **F**, Spike phase histograms relative to ongoing gamma-frequency oscillations,
calculated from the fraction of total spike counts. **G**, Sustained activation index (top), modulation
index (middle) and vector length (bottom). **H**, Instantaneous amplitude of the Hilbert transform
during theta photo-activation (1 mW) overlaid across experiments (grey traces, n = 12), black
represents the mean and dark grey the SEM. Dotted lines highlight peaks and troughs in the light
waveform. **I**, Intracellular current-clamp recordings from pyramidal cells in aCSF, showing
responses to current steps (left) and hyperpolarisation in response to PV+ interneuron photo-
activation (right; n = 4).

**Figure 7**: Sustained photo-excitation of SST+ interneurons decreases the power and increases
the frequency of Cch-induced gamma oscillations, but can also induce high-frequency
oscillations. **A-B**, Representative LFP recordings from CA3 area illustrating effect of SST+
interneurons photo-excitation (155 μW) on gamma oscillations (**A**), along with the respective
power spectra (**B**; arrows indicate peaks in the power spectra). **C-D** Time course of normalised
power area (**C**) and peak frequency (**D**), each calculated in 0.5 second bins across experiments (n =
12). **E**, Mean changes in normalised power area. **F**, Changes in mean peak frequency; rMANOVA:
F(1.05, 11.59) = 15.05, p = 0.002. **G-H**, Normalised power area (**G**) and changes in peak frequency
(**H**) plotted against light intensity (n = 12). **I**, Strong and sustained blue light illumination (5.5 mW),
that does not cause the collapse of Cch-induced oscillations, induces high-frequency oscillations,
as seen in LFP traces (top) and normalised wavelet spectrum (bottom). **J**, Normalised power during
SST+ interneurons cell photo-activation (n = 31). **K**, Peak frequency of oscillations that were not
abolished during strong light illumination (n remaining = 16/31: n = 4 at 5.5 mW and n = 12 at 2.2
mW); rmANOVA: F(1.03, 15.39) = 31.45, p < 0.001. Changes in peak frequency were analysed using rmANOVA, followed by post-hoc paired t-tests with correction for multiple comparisons.

Responses to laser illumination (approx. 18.6 mW) in SST-cre mice expressing ArchT-GFP with and without the presence of Cch. Orange squares represent the bottom sections of LFP that were magnified. M, Changes in normalised power area calculated in 1 second bins across experiments (n = 3) in aCSF only. Dotted lines indicate the duration of laser illumination. *p < 0.05, **p < 0.01, ***p < 0.001, n.s. p >= 0.05. Solid brackets represent paired t-tests and standalone star symbols represent one-sample t-test versus normalised baseline. Grey lines represent single experiments.

Figure 8: Photo-activation of SST+ interneurons induces de novo oscillations in the absence of Cch. A-B, Representative LFP recordings from CA3 illustrating induction of high-frequency oscillations by step (A) and theta-modulated (B) blue light illumination (10 mW), as seen in LFP traces (top) and normalised wavelet spectrum (bottom). C, Change in log power compared to baseline during step (n = 16) and theta-modulated blue light illumination (n = 17). D, Peak frequency of the de novo oscillations is higher when induced by theta when compared to tonic stimulation; two-sample t-test, ***p < 0.001. E-F, Power area (E) and peak frequency (F) plotted against light intensity of theta photo-activation (n = 12). The black line is the mean response and the dark-grey shaded area represents SEM. G-H, pharmacology of de-novo oscillations induced by sinusoidal blue light illumination in SST-cre mice expressing ChR2-mcherry (1 - 10 mW).

Representative LFP recording in CA3 before (top) and after (bottom) application of G.i) iGluR blockers and H.i) GABA_A blockers. Power-area change before (control) and after application of G.ii) iGluR blockers and H.ii) GABA_A blockers. iGluR blockers used: 20 μM CNQX, 40 μM AP5, n = 3; 10 μM CNQX, 20 μM AP5, n = 1; 20 μM CNQX, n = 1; 3mM kynurenic acid, n = 3. GABA_A blockers: 20 μM Bicuculline, n = 2; 20 μM Gabazine, n = 1. I, Representative multi-unit recordings
of SST+ interneuron activity during step photo-excitation, showing average spike waveform, autocorrelation, phase and step histogram. **J-K** Mean spike time histograms during step illumination (*J*) and sinusoidal theta stimulation (8 Hz; *K*), calculated from the fraction of total spike counts in each bin for each clustered neuron. **L**, Spike phase histograms relative to ongoing gamma-frequency oscillations, calculated from the fraction of total spike counts. **M**, Sustained activation index (top), modulation index (middle) and vector length (bottom).

**Figure 9: Computational model of the network effects of optogenetic modulation of interneuron activity.** **A**, Schematic of connectivity between excitatory cells (E) and PV+ and SST+ interneurons. E and PV+ cells were modelled using Wilson-Cowan equations and SST+ using equations derived from quadratic integrate-and-fire neurons, which can show intrinsic oscillations (~). **B**, Activity patterns observed in the 3 populations of cells when external drive to E cells is 10, and following inhibition (Arch; top) or excitation (ChR2; bottom) of either PV+ (left) or SST+ (right) populations. **Inset left**: expansion of traces highlighted by the dashed box during the baseline period, showing temporal order of peak activity. **Inset right**: expansion of E cell activity during the period shown by the dashed lines, showing small and fast oscillations. **C**, Corresponding changes in peak power (top) and oscillation frequency (bottom), following manipulation of the external drive to PV+ (left) or SST+ (right) populations. **D**, Plot of spike rate of E cells versus PV+ cells for the manipulations of PV+ cells (left) and plot of spike rate of E cells versus SST+ cells for manipulation of SST+ cells (right). The colour of each marker represents the frequency of the network oscillation and the size of the marker reflects peak power. Black lines join the points showing spike rates during the baseline conditions. **E**, Delays between the peak in E cell activity and the peak in activity for PV+ and SST+ cells across different manipulations. The size of the marker reflects the corresponding spike rate. **F**, Light-induced decreases in both excitatory drive and presynaptic release, aimed at
mimicking laser stimulation of Arch, can lead to the collapse of the oscillations when applied to either the PV+ (left) or SST+ (right) populations.

### Tables

|         | Synaptic weights | Synaptic time constants (ms) |
|---------|------------------|-------------------------------|
| post    |                  |                               |
| pre     | $E$              | $PV$                          |
|         |                  | $SST$                         |
| $E$     | 10               | 30                            |
|         | 10               | 3                             |
|         | 10               | 5                             |
| $PV$    | -15              | -10                           |
|         | 7                | 7                             |
| $SST$   | -15              | -10                           |
|         | 10               | 10                            |

Table 1. Synaptic parameters. $E$ – excitatory neurons; $PV$ - PV+ inhibitory neurons; $SST$ – SST+ inhibitory neurons.
null
A. Tonic Theta

B. Frequency Distribution

C. Log Normal Power Change

D. Peak Frequency Distribution

E. Theta Power Area

F. Light Intensity (mW)

Gi. SST-ChR2 (aCSF)

Gii. Norm. Power

Hi. SST-ChR2 (aCSF)

Hii. Norm. Power

I. Spike Count

J. Activation Index

K. Mod. Index

L. Vector length

M. aCSF only

N. Control

O. iGluR Blocker

P. GABAAR Blocker

Q. Gi Gii Hii

R. Spike Count

S. Time (ms)

T. Spike Count

U. Time (s)

V. Spike Count

W. Time (s)

X. Spike Count

Y. Time (s)
