Network mechanisms of theta related neuronal activity in hippocampal CA1 pyramidal neurons

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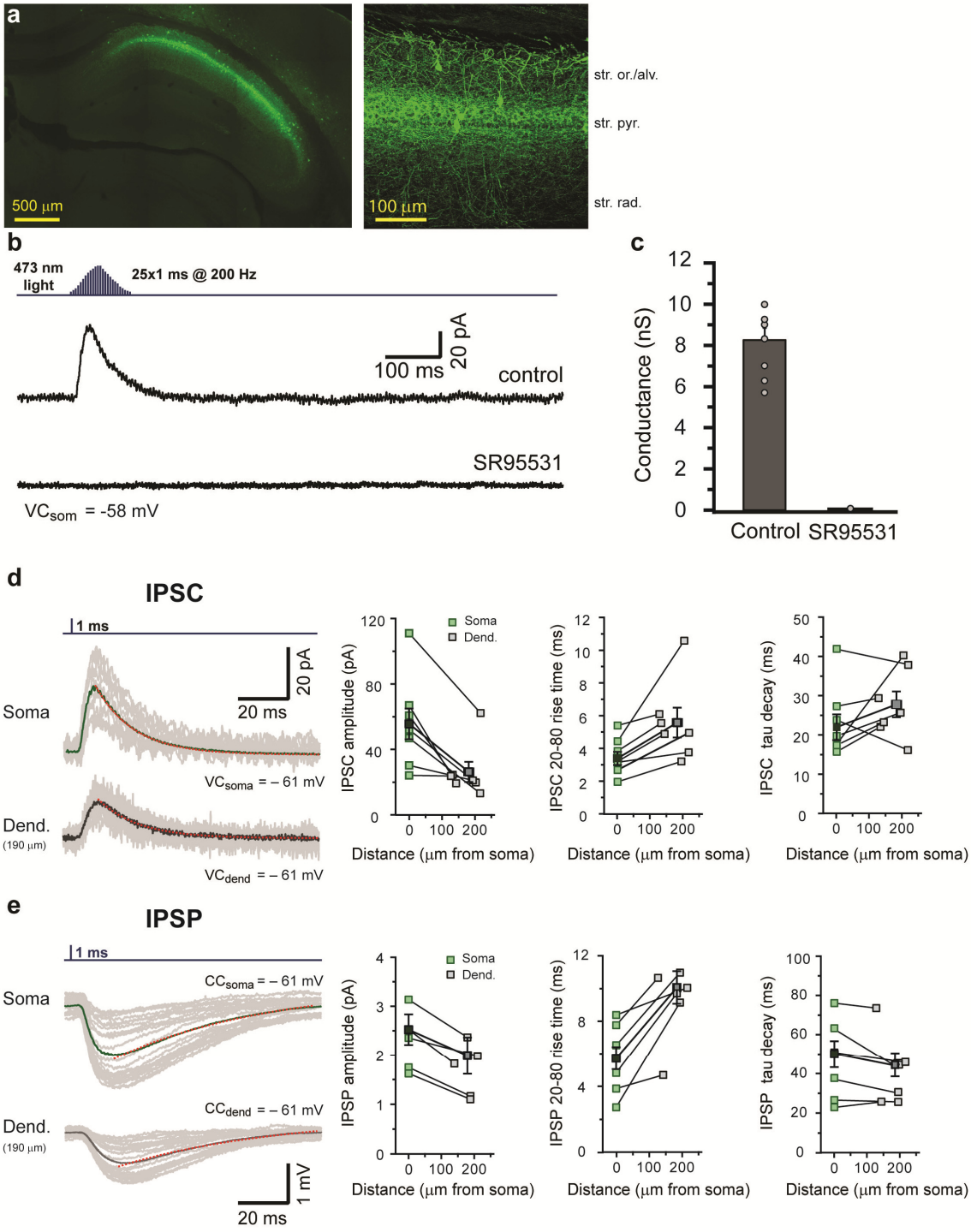
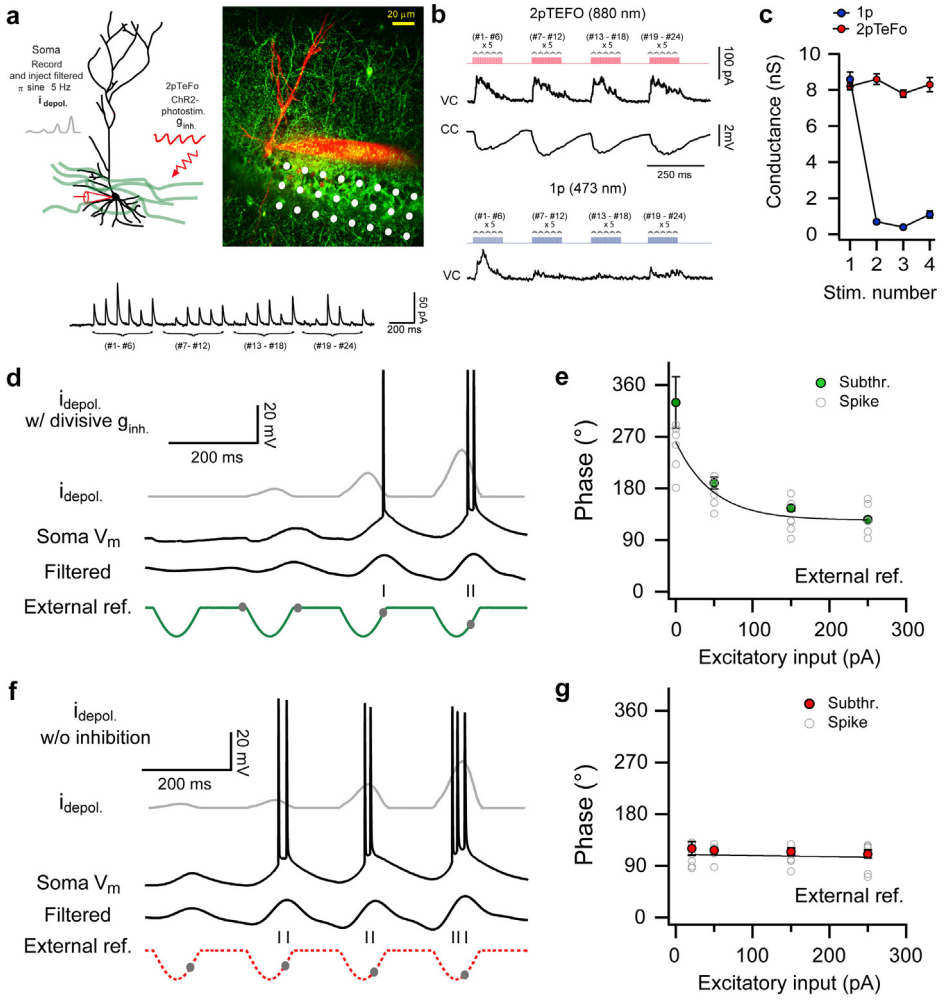
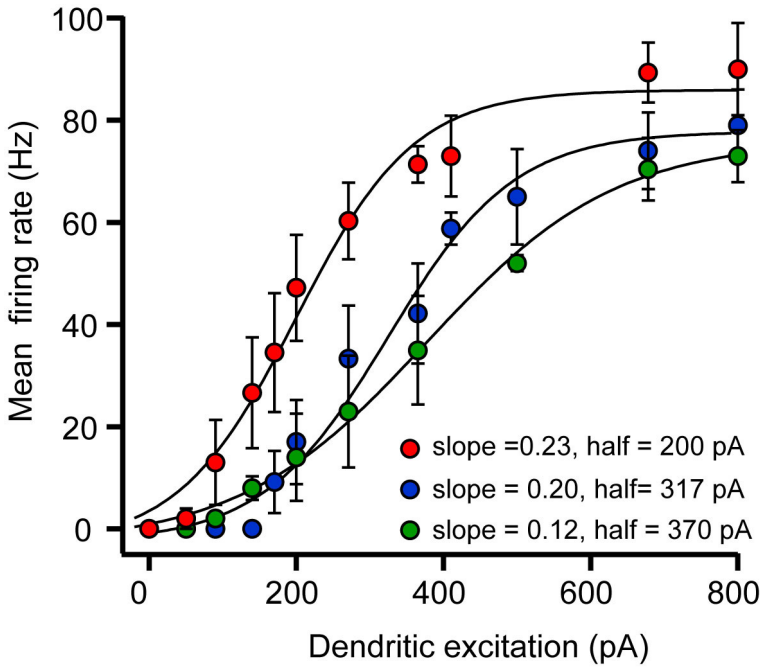
Supplementary Figures S1 to S6



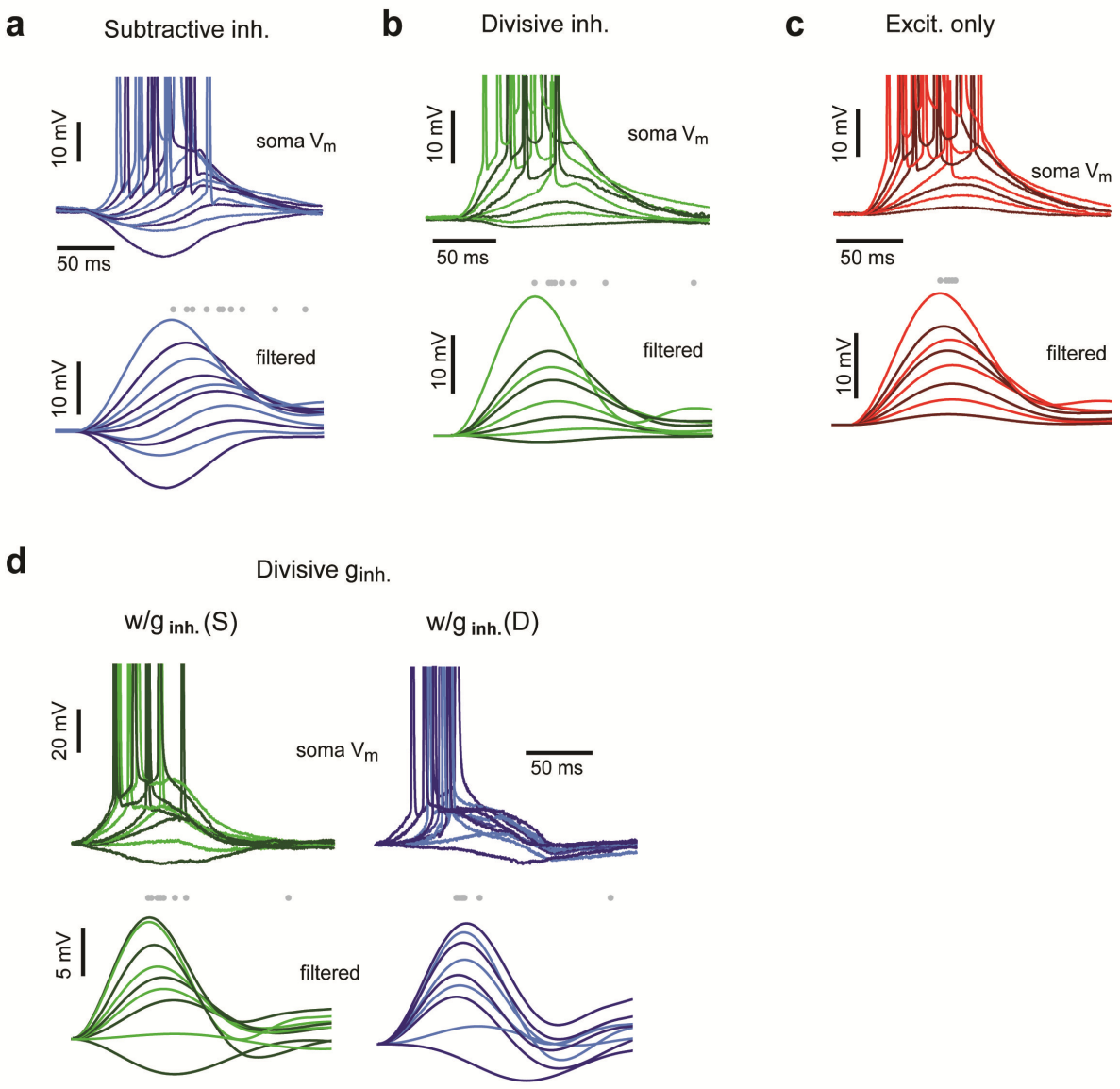
Supplementary Figure 1 Properties of inhibition evoked by ChR2-photostimulation on CA1 pyramidal neurons in PV-Cre mice. (**a**) Left, low magnification confocal stack image of a dorsal hippocampal slice from a PV-Cre mice injected with rAAV-FLEX-*rev*-ChR2-GFP. Right, higher magnification confocal stack image from the hippocampal CA1 region. (**b**) Synchronous ChR2-photostimulation-evoked (25 pulses with a 3 ms interval) current recorded at the soma at a depolarized holding potential in voltage-clamp mode in control and in the presence of GABA-A receptor antagonist (SR95531, 20 μM). (**c**) Summary bar graph of inhibitory synaptic conductance evoked by ChR2-photostimulation in control and the presence of GABA-A receptor antagonist (n = 9 in control and n = 4 in SR95531). Symbols: individual recordings; bars: mean ± s.e.m. (**d**) Left, ChR2-photostimulation-evoked IPSCs (10 individual traces: grey, average: black, red dotted line: exponential fit) recorded at the soma and at the dendrite. Right, summary data of IPSC kinetics (n = 8) at the somatic and dendritic locations. Lines connect individual soma-dendrite recordings. (**e**) Left, IPSPs (10 individual traces: grey, average: black, red dotted line: exponential fit) evoked by ChR2-photostimulation recorded at the soma and at the dendrite. Right, summary data of IPSP kinetics (n = 8) at the somatic and dendritic locations.



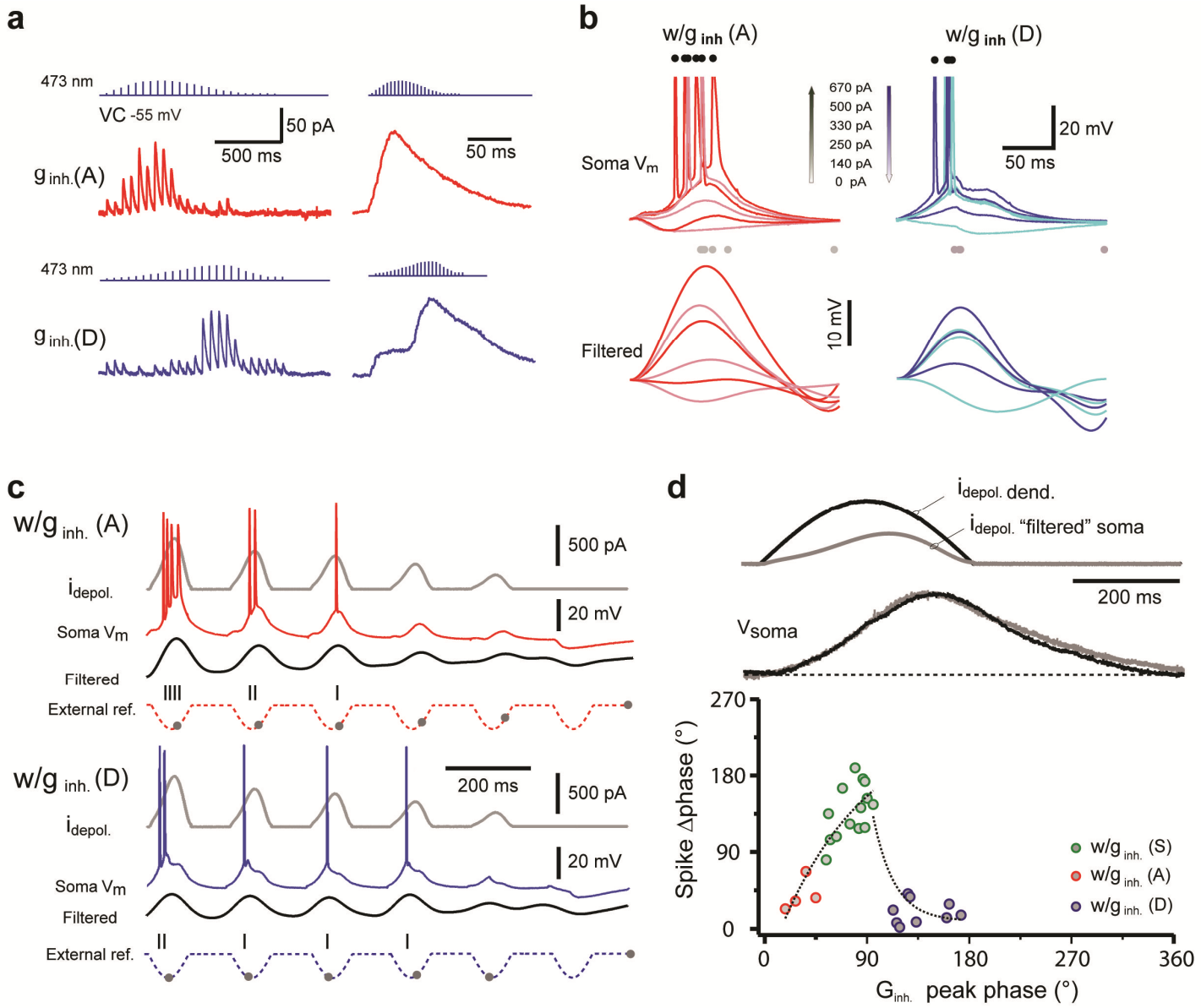
Supplementary Figure 2 Repetitive ChR2-photostimulation of PV+ interneurons using two-photon temporal focusing. (**a**) Left, schematic of the experimental configuration. A somatic whole-cell electrode was used to inject a filtered depolarizing sine wave-shaped current, while the corresponding voltage responses were recorded in the absence or presence of inhibitory conductance evoked by two-photon temporal focusing ChR2-photostimulation within the cell body layer of hippocampal CA1 region. Right, two-photon stack of a CA1 pyramidal neuron (red: Alexa 594) and ChR2-GFP+ fibers (green) concentrated in the pyramidal layer in a hippocampal slice from a PV-Cre mouse injected with rAAV-FLEX-*rev*-ChR2-GFP. Gray dots: stimulation grid used for 2pTeFo ChR2-photostimulation. Bottom, individual IPSCs evoked by 2pTeFo photostimulation (1-24 locations, 100 ms interval). (**b**) Red tickmarks indicate timing of laser pulses used for repeated (four cycles) photostimulation: four bursts, each burst composed of 30 stimuli (1 ms duration with a 3 ms interval, six consecutive locations, repeated five times). Different clusters of six locations were used for consecutive bursts. Inhibitory postsynaptic currents (*VC*: voltage-clamp, red) and inhibitory postsynaptic potentials (*CC*: current-clamp, red) evoked by repeated stimulation. Note that 2pTeFo photostimulation evokes reliable non-desensitizing responses during repeated stimulation. Rapidly desensitizing current trace evoked by one-photon (*1p*, blue) photostimulation is also shown. (**c**) Summary of response magnitude versus burst stimulus number during repeated stimulation evoked by 2pTeFo (n = 4) and by single-photon photostimulation (n = 3). (**d**) Increasing levels of 5-Hz filtered depolarizing current injection (upper gray; 0, 50, 150, 250 pA) paired with four cycles of sine wave-shaped repeated 2pTeFo photostimulation of PV+ interneurons. Somatic membrane potential (*Soma V_m*), 3-8 Hz bandpass-filtered V_m (*Filtered*) and approximate soma conductance (e.g. external LFP reference, dark green) waveforms are shown. AP latency and timing of the subthreshold peak depolarization advance with increased dendritic current (grey dots: depolarization peak). (**e**) Summary of mean action potential phase (open gray circles) and mean subthreshold potential peak time (mean ± s.e.m, solid green circles, n = 4 cells) plotted versus amplitude of depolarizing current injection paired with perisomatic inhibition (n = 4 cells) evoked by 2pTeFo photostimulation. Spike times are fitted with an exponential. (**f**) Increasing levels of filtered dendritic depolarizing current injections (upper gray; 25, 50, 150, 250 pA) delivered without inhibition. Somatic membrane potential (*Soma V_m*), 3-8 Hz bandpass filtered V_m (*Filtered*) and missing soma conductance (e.g. external reference LFP, red dashed) waveforms are shown. AP latency and timing of the subthreshold peak depolarization advance only slightly with increased depolarizing currents (grey dots indicate somatic depolarization peak). (**g**) Summary of mean action potential phase (open gray circles) and mean subthreshold potential peak time (solid red circles, n = 4 cells) plotted versus amplitude of current injection for current injection alone (n = 4 cells). Spike times are fitted with an exponential.



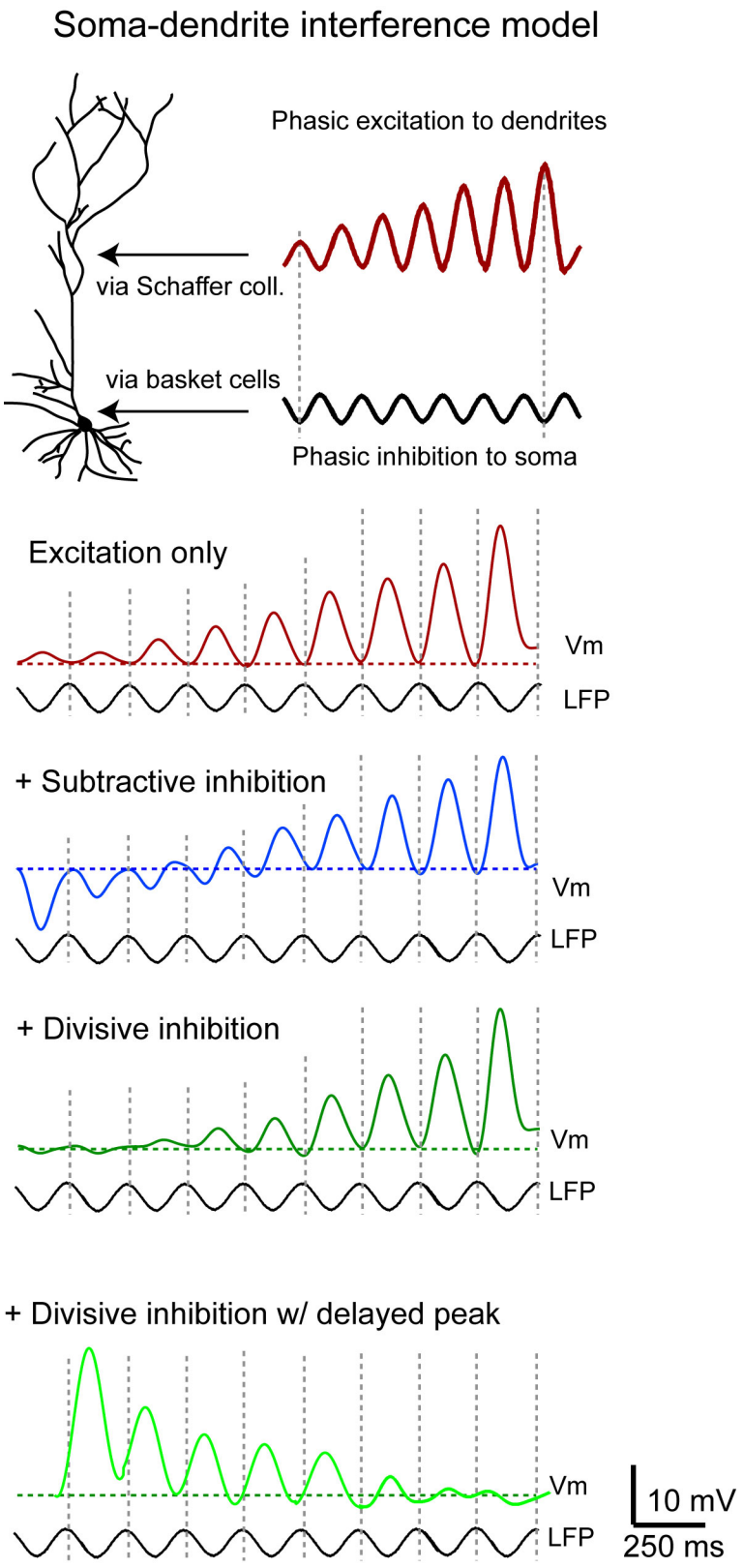
Supplementary Figure 3 Action potential frequency versus input relationships. Mean *F-I* curves are shown for hyperpolarizing current injections (blue, n = 6 cells), somatic conductance changes (green, n = 6 cells) or no inhibition (red, n = 6 cells, mean ± s.e.m). Curves were fit by a sigmoid function to determine the level of rightward shift (half) and a linear function to give the gain changes (slope).



Supplementary Figure 4 Soma-dendrite interference induces phase precession. (**a**) *Dendritic excitation plus somatic current injection*. Superimposed individual traces of hyperpolarizing 5 Hz sine wave shaped current injections (100 pA constant amplitude) into the soma paired with depolarizing 5 Hz sine wave-shaped current injections into the distal apical dendrites. As the dendritic current amplitude is increased (0, 40, 75, 130, 200, 300, 500, 670, 1300 pA) the action potential latency (upper) and timing of the subthreshold peak depolarization (lower) advances (grey dots indicate depolarization peak). Concatenation of the traces are shown in *Fig. 2a*. (**b**) *Dendritic excitation plus somatic conductance stimulation*. Superimposed individual traces of increasing dendritic depolarizing current injection (0, 40, 75, 130, 200, 300, 500, 670, 1300 pA) paired with 5 Hz sine shaped ChR2-photostimulation of PV+ interneurons. AP latency (upper) and timing of the subthreshold peak depolarization (lower) advances with increased dendritic current (grey dots indicate depolarization peak). Concatenation of the traces are shown in *Fig. 2b*. (**c**) *Dendritic excitation only.* Superimposed individual traces of increasing dendritic depolarizing current injection (40, 75, 130, 200, 250, 300, 500, 670 pA) is delivered alone without somatic inhibition. AP latency (upper) and timing of the subthreshold peak depolarization (lower) advance only slightly with increased dendritic current (grey dots indicate depolarization peak). Concatenation of the traces are shown in *Fig. 2c*. (**d**) Superimposed individual traces of 5 Hz filtered sine wave-shaped depolarizing current injections into the soma paired with 5 Hz symmetrical ($g_{inh}(S)$, green traces on left) or delayed ($g_{inh}(D)$, blue traces on right) inhibitory conductance profiles evoked by 2pTeFo photostimulation. As the depolarizing current amplitude is increased (left), the AP latency (upper, black dots) and timing of the subthreshold peak depolarization advance (lower, grey dots indicate depolarization peak) when paired with symmetrical temporal profile of inhibition. When depolarizing current amplitude is decreased, and is paired with a delayed inhibitory conductance profile at the soma, the AP latency (upper) and timing of the subthreshold peak depolarization (lower) stay advanced. Traces are from recording shown in *Fig. 4a-c*.



Supplementary Figure 5 Phase of inhibitory inputs versus phase precession. (**a**) Individual (left, 1-25 locations with 100 ms interval) and synchronous (right, 1-25 locations with 3 ms interval) inhibitory postsynaptic currents (*VC*: voltage-clamp) evoked by one-photon ChR2-photostimulation. Blue tickmarks indicate the timing and relative intensity of 473 nm laser pulses used to evoke inhibitory conductance profiles that were advanced ($g_{inh}(A)$, red) or delayed in time ($g_{inh}(D)$, blue). (**b**) 5 Hz filtered sine wave-shaped depolarizing current injections into the soma paired with 5 Hz advanced- ($g_{inh.}(A)$, red) or delayed ($g_{inh.}(D)$, blue) photostimulation of PV+ interneurons. As the depolarizing current amplitude is increased, the AP latency (upper) and timing of the subthreshold peak depolarization (lower) move forward moderately (grey dots indicate depolarization peak, black dots indicate AP peak) when paired with advanced inhibition (red). When decreasing levels of depolarizing currents were paired with a delayed inhibitory conductance profile (blue), the AP latency (upper) and timing of the subthreshold peak depolarization (lower) stay advanced. (**c**) Concatenation of depolarizing current injections (upper dark grey), somatic membrane potential (*Soma V_m*), 3-8 Hz bandpass-filtered V_m (black) and external reference (dashed black lines, representing average population inhibitory waveform) for forward- (red) and backward-shifted (blue) shifted inhibition. Tickmarks indicate AP location and grey dots represent the phase of subthreshold peak depolarization. (**d**) Plot of peak phase of inhibitory conductance versus maximum change in spike phase. Note that inhibition delayed in time (peak $g_{inh.}$ phase > 90°, blue circles, mean peak g_{inh}: 137 ± 7°, n = 9) overlaps with the peak of the filtered depolarization waveform at the soma and reduces the range of spike phase modulation (Δspike phase: 19 ± 5°, n = 9) by effectively blocking spiking later in the cycle. Inhibition with peak phase centered around 90° (mean peak $g_{inh.}$: 79 ± 4°, n = 14) induces maximum phase change (green circles, Δspike phase: 138 ± 8°, n = 14). The range of spike phase modulation is reduced with temporally advanced inhibition (peak ginh. phase < 45° red circles, mean peak g_{inh}: 32 ± 6°, n = 4) and spiking is confined to later in the cycle (Δspike phase: 42 ± 11°, n = 4). Traces on the top are dendritic sine wave (black, $i_{depol.\ dend.}$) and somatic filtered sine wave (grey, $i_{depol.\ soma}$) current waveforms an corresponding somatic membrane potential responses (V_{soma}).



Supplementary Figure 6 Soma-dendrite interference model using actual perisomatic inhibitory conductance with modulated temporal profile best captures theta activity. Sketch of interference model used showing the input patterns (upper) and the resulting somatic subthreshold potentials (V_m, DC-10 Hz filtered) for the four experimental conditions. Trace labeled "LFP" is input sine wave shown for reference.