Cell Reports, Volume *29*

Supplemental Information

The Spatial Extent of Optogenetic Silencing in Transgenic Mice Expressing Channelrhodopsin in Inhibitory Interneurons

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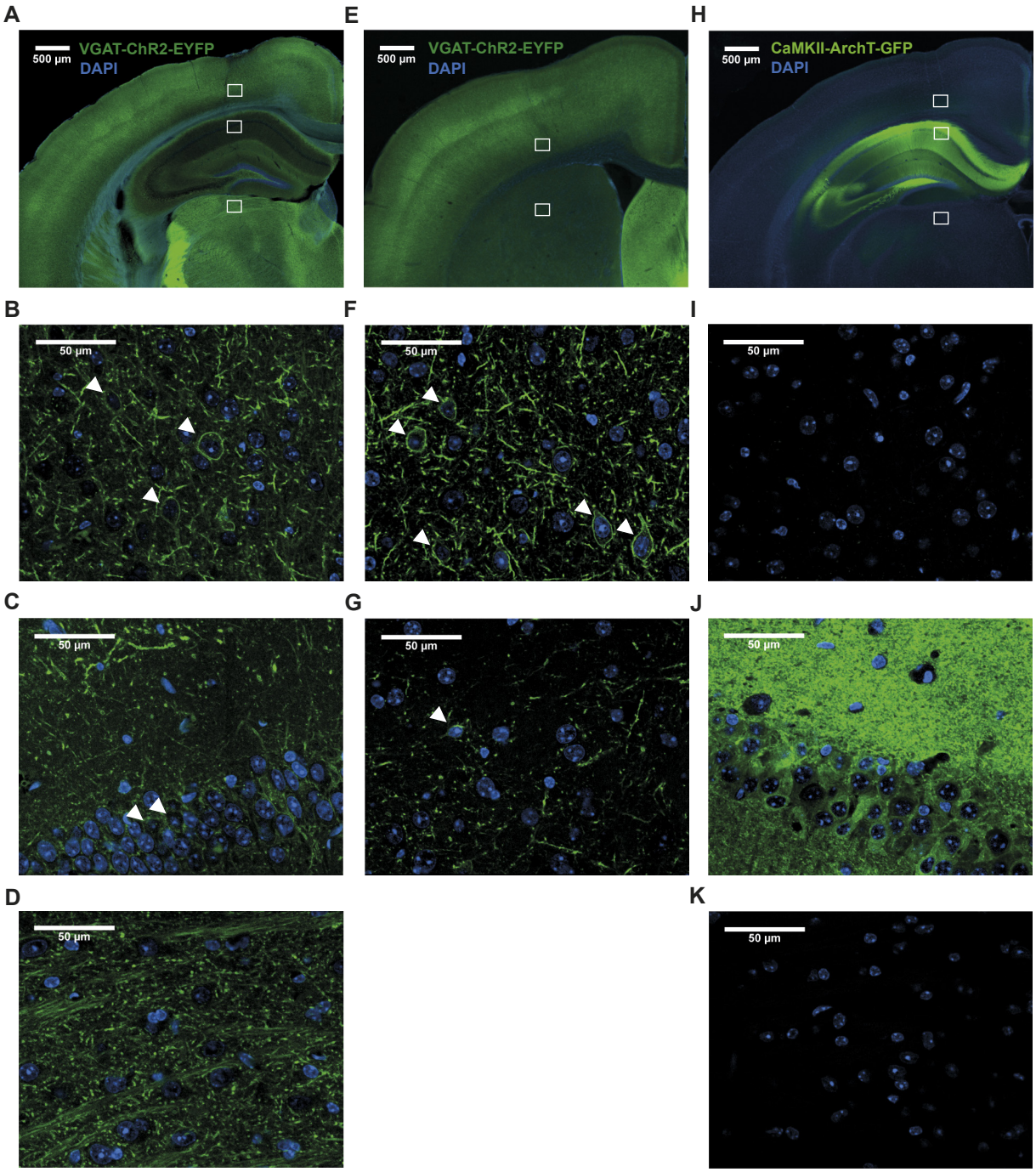

Figure S1, Opsin expression in brain areas that were recorded from VGAT-ChR2 and ArchT-expressing mice, related to Figures 1-3 and 6. ***A***, Coronal brain section from a VGAT-ChR2 mouse showing ChR2-EYFP expression in cortex, hippocampus and thalamus (areas recorded from experiments described in Figures 1-2). ***B-D***, High magnification confocal images of areas defined by white rectangles in ***A***, showing ChR2-EYFP positive cell bodies (white arrows) and axon fibers in cortex (***B***), CA1 pyramidal layer (***C***) and thalamus (***D***). ***E***, Coronal section showing ChR2-EYFP expression in cortex and striatum (areas recorded from experiments described in Figure 3). ***F-G***, High magnification images of areas defined by white rectangles in ***E***, showing ChR2-EYFP positive cell bodies and axon fibers in cortex (***F***) and striatum (***G***). ***H***, Coronal section from a mouse expressing ArchT-GFP in hippocampus. ***I-K***, High magnification images corresponding to white rectangles in ***H***, showing strong ArchT-GFP expression in neurons in CA1 (***J***), but not in the overlying cortex (***I***) or the subjacent thalamus (***K***).

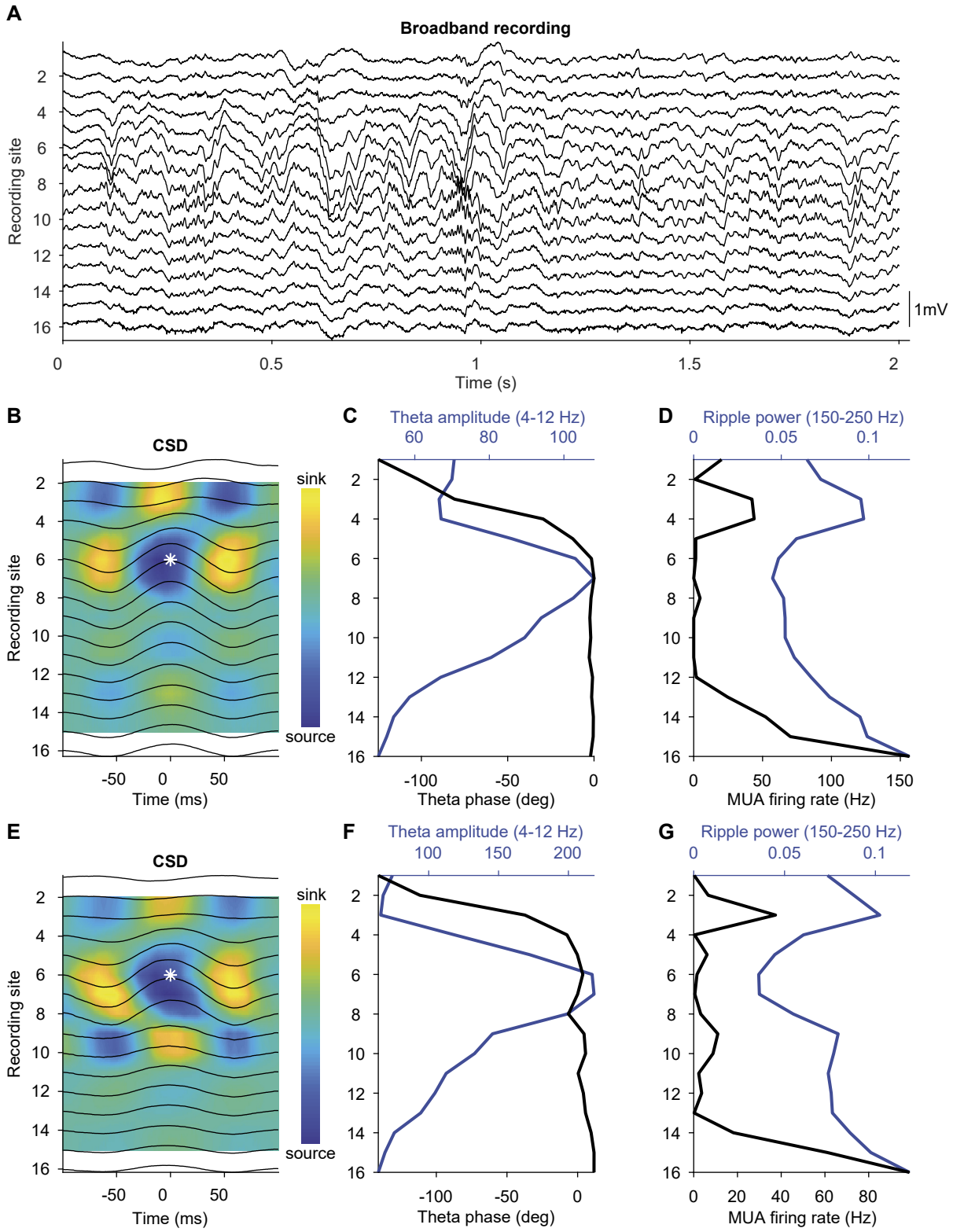

Figure S2, Signatures of hippocampal activity used for identifying recording positions, related to Figures 1, 2, 6 and STAR Methods. ***A***, Example broadband recording from a 16-channel optoprobe with recording sites in hippocampus and thalamus (as in Figure 1C). ***B-D***, Hippocampal activity signatures for one example recording. ***B***, Averaged peak-triggered theta (4-12 Hz) oscillations (black traces) and the corresponding CSD (color plot). White asterisk indicates a current source in the hippocampal fissure. ***C***, Theta phase (black) and theta amplitude (blue) across recording sites reveals the largest amplitude on channel 7 indicating the location of the hippocampal fissure. ***D***, Ripple power (blue) and mean multi-unit firing rate (black) across recording channels. A local maximum can be observed at channels 3-4, indicating the pyramidal layer. ***E-G***, Same as in ***B-D*** but for another example recording.

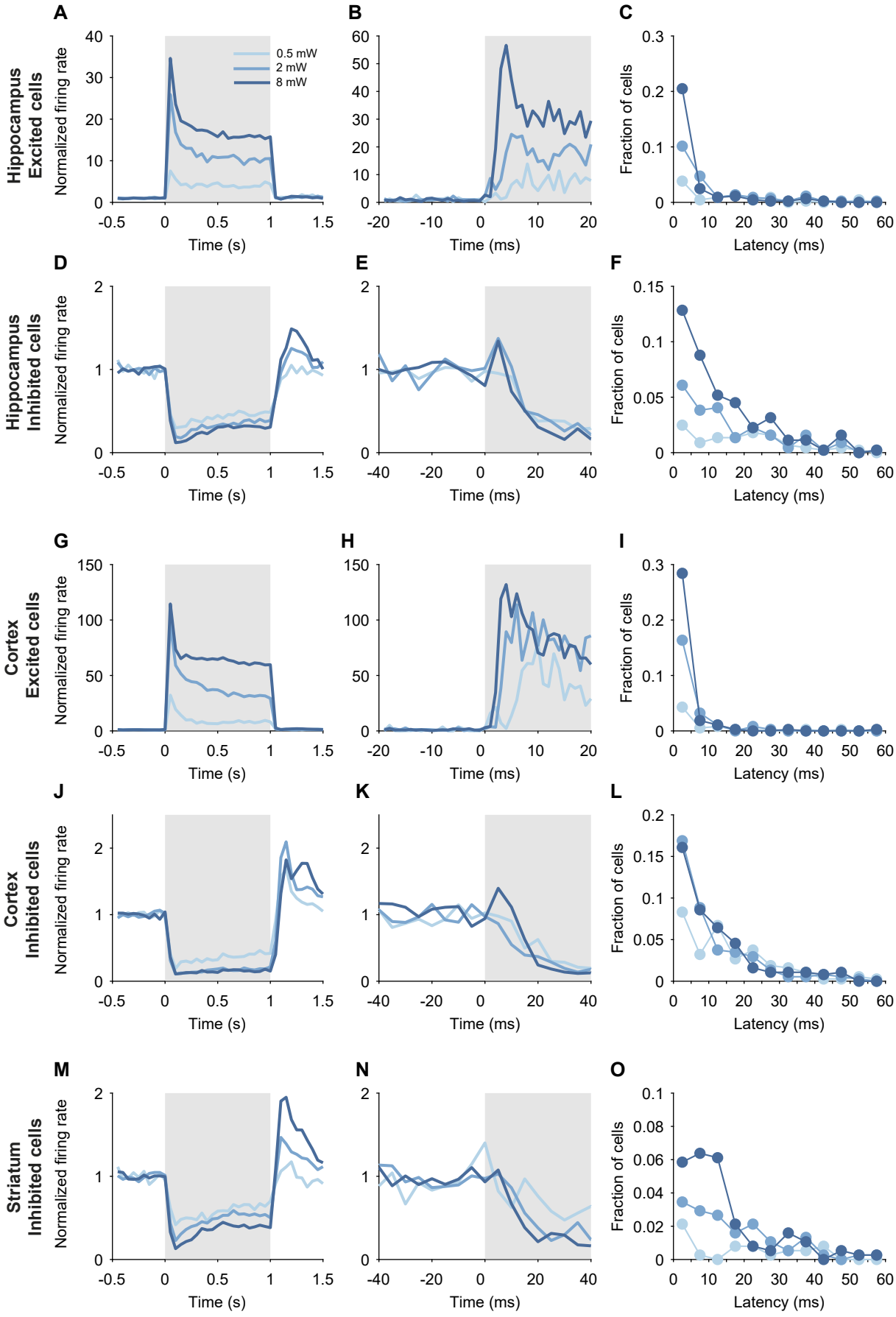
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
Hippocampus Excited cells
Hippocampus Inhibited cells
Cortex Excited cells
Cortex Inhibited cells
Striatum Inhibited cells
Normalized firing rate
Fraction of cells
0.5 mW
2 mW
8 mW
Time (s)
Time (ms)
Latency (ms)

Figure S3, Time course and latency of excitatory and inhibitory responses in VGAT-ChR2 mice, related to Figures 1-4. ***A***, Average normalized firing rates of significantly excited cells recorded in the hippocampus during light stimulation at different intensities (bin size: 50 ms). ***B***, Same as in ***A*** but with a finer temporal resolution (bin size: 1 ms) to show the response at light onset. ***C***, Distribution of response latencies (see STAR Methods) of excited hippocampal neurons for different light intensities. Tick marks on the x-axis represent the edges of 5-ms bins. Most neurons responded at a short latency of < 5 ms. ***D-F***, Same as in ***A-C*** but for hippocampal neurons that were significantly inhibited (bin size in ***E***: 5 ms). ***G-L***, Time course and latency distribution of excited (***G-I***) and inhibited (***J-L***) neurons in the parietal and somatosensory cortex, shown as in ***A-F***. ***M-O***, Mean normalized firing rate (***M, N***) and latency distribution (***O***) of significantly inhibited cells recorded in striatum during light stimulation at different intensities.

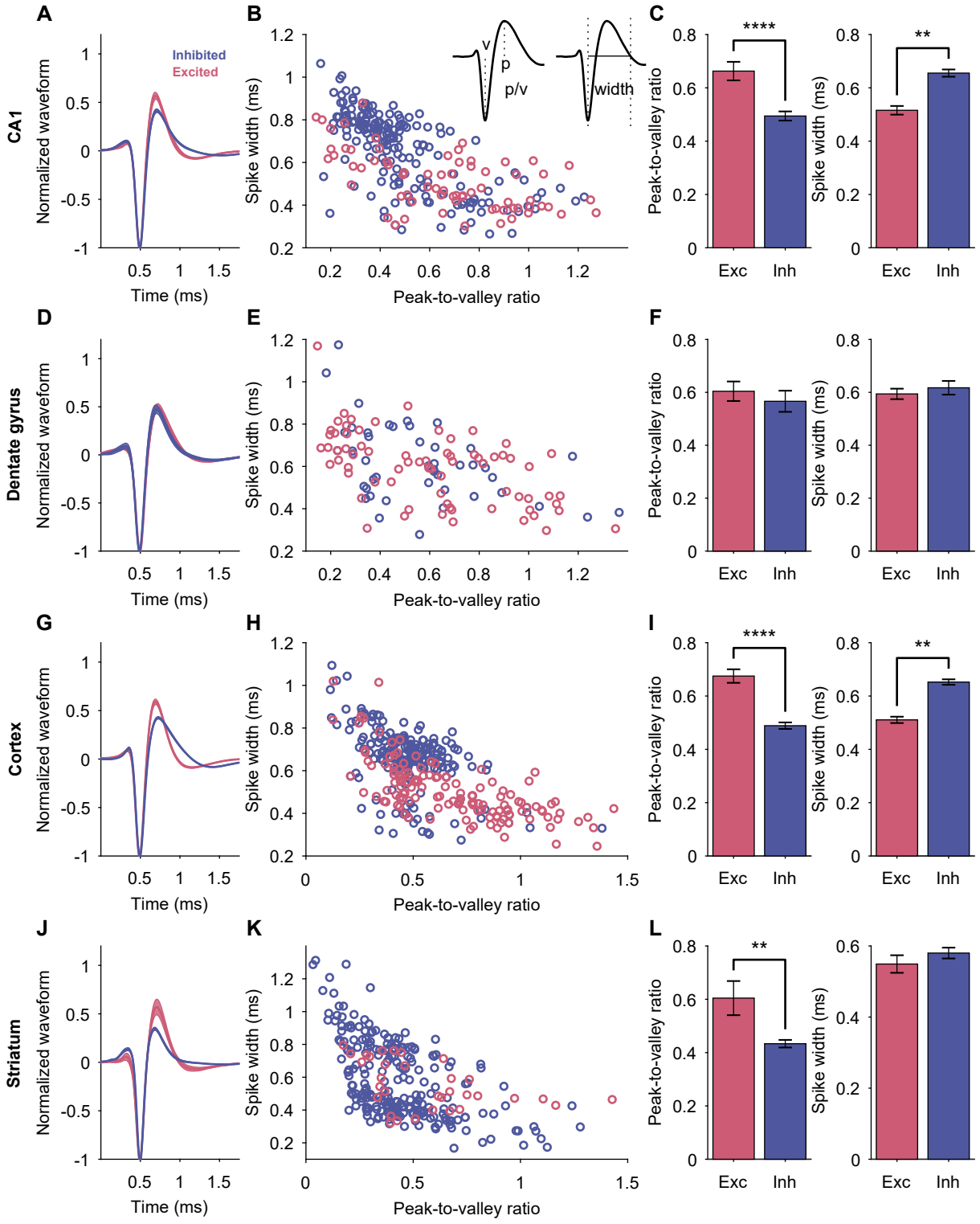

Figure S4, Action potential waveforms of excited and inhibited neurons in VGAT-ChR2 mice, related to Figures 1-4. ***A***, Normalized waveforms of excited and inhibited cells recorded in the CA1 region of the hippocampus. ***B***, Scatter plot of peak-to-valley ratio against spike width (see example waveforms in inset) of significantly excited and inhibited cells in CA1. ***C***, Comparison of peak-to-valley ratio (left) and spike width (right) reveals that waveforms of excited CA1 neurons are narrower and have a larger peak-to-valley ratio, suggesting that they are inhibitory interneurons. ***D-L***, Waveform analyses as in ***A-C*** of cells recorded in dentate gyrus (***D-F***), somatosensory and parietal cortex (***G-I)*** and striatum (***J-L***). Error bars and shaded areas represent the mean ± s.e.m. over neurons.**p<0.01; ****p<0.0001

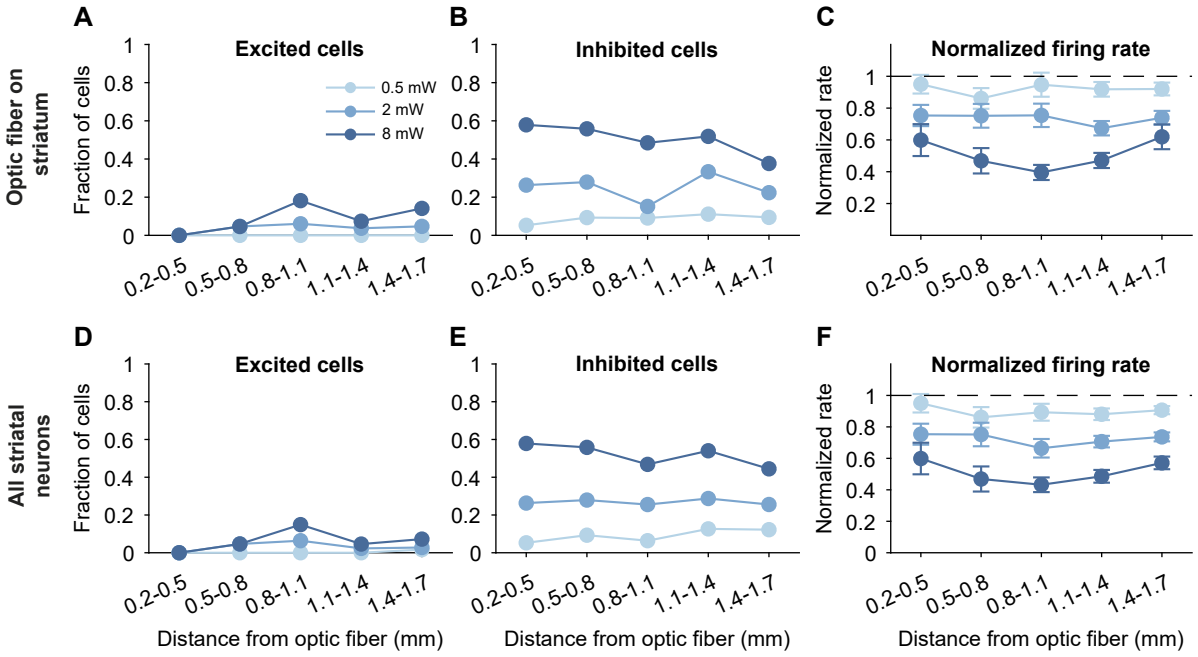

Figure S5, Excitation and inhibition in striatal cells from VGAT-ChR2 mice, related to Figure 3. ***A-C***, Fraction of significantly excited (***A***) and inhibited (***B***) cells and normalized firing rates (***C***) of cells recorded in the striatum with the optic fiber on the optoprobe located on top of the striatum (n=234 cells from 3 mice). ***D-F***, same as for ***A-C*** but for all recorded striatal neurons combined, including those shown in Figure 3 (n=376). Error bars and shaded areas represent the mean ± s.e.m. over neurons.

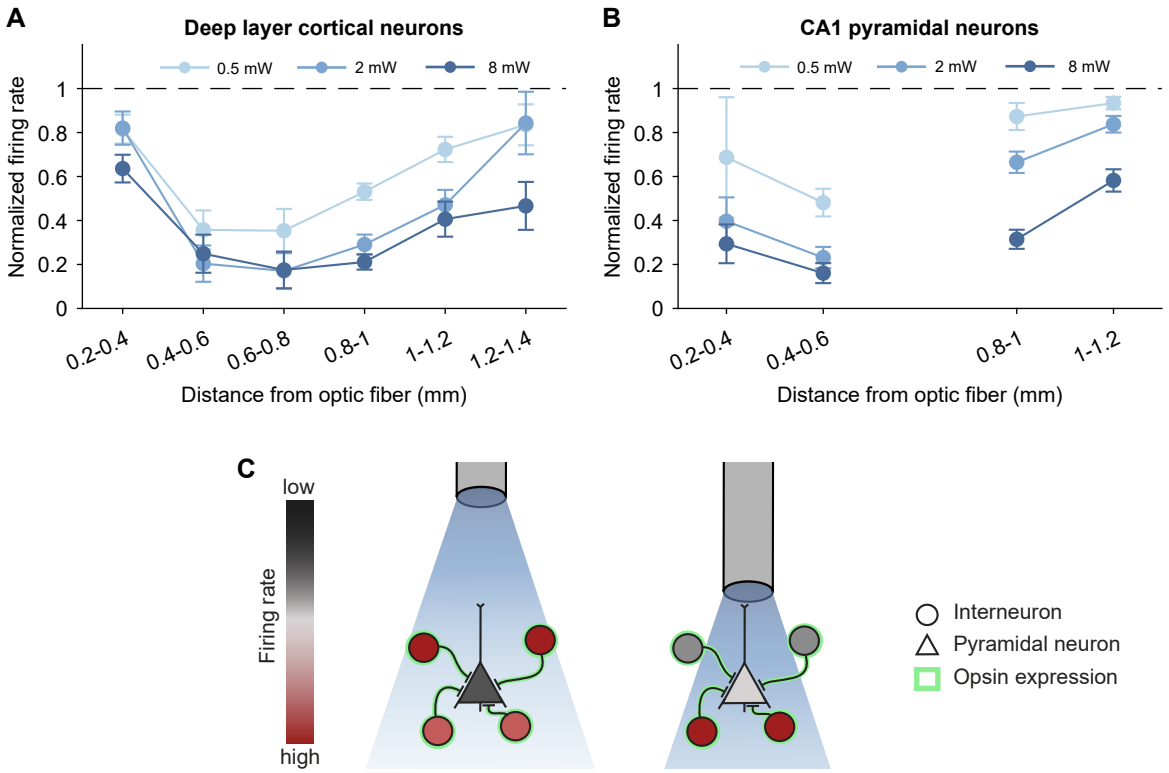

Figure S6, Inhibition in specific neuronal populations in VGAT-ChR2 mice as a function of distance from the optic fiber, related to Figure 4. ***A***, Normalized firing rates of putative excitatory cells recorded in deep layers of the parietal cortex (≥ 500 µm below brain surface) and the somatosensory cortex (≥ 700 µm below brain surface) as a function of distance from the optic fiber. ***B***, Normalized firing rate of putative pyramidal cells recorded in the pyramidal layer (±100 µm) of CA1, as a function of distance from optic fiber. For both neuronal populations, inhibition was weaker right below the fiber compared to slightly further away (1st vs 2nd distance bin; main effect of distance in a distance X intensity ANOVA: $p<0.0001$ for both deep layer cortical neurons and CA1 pyramidal layer neurons). Error bars represent the mean ± s.e.m. over neurons. ***C***, Schematic explanation for why inhibition is weaker closer to the optic fiber. Because of the horizontal spread of light in brain tissue, a larger proportion of a neuron's inhibitory input population is recruited when the optic fiber is further away.

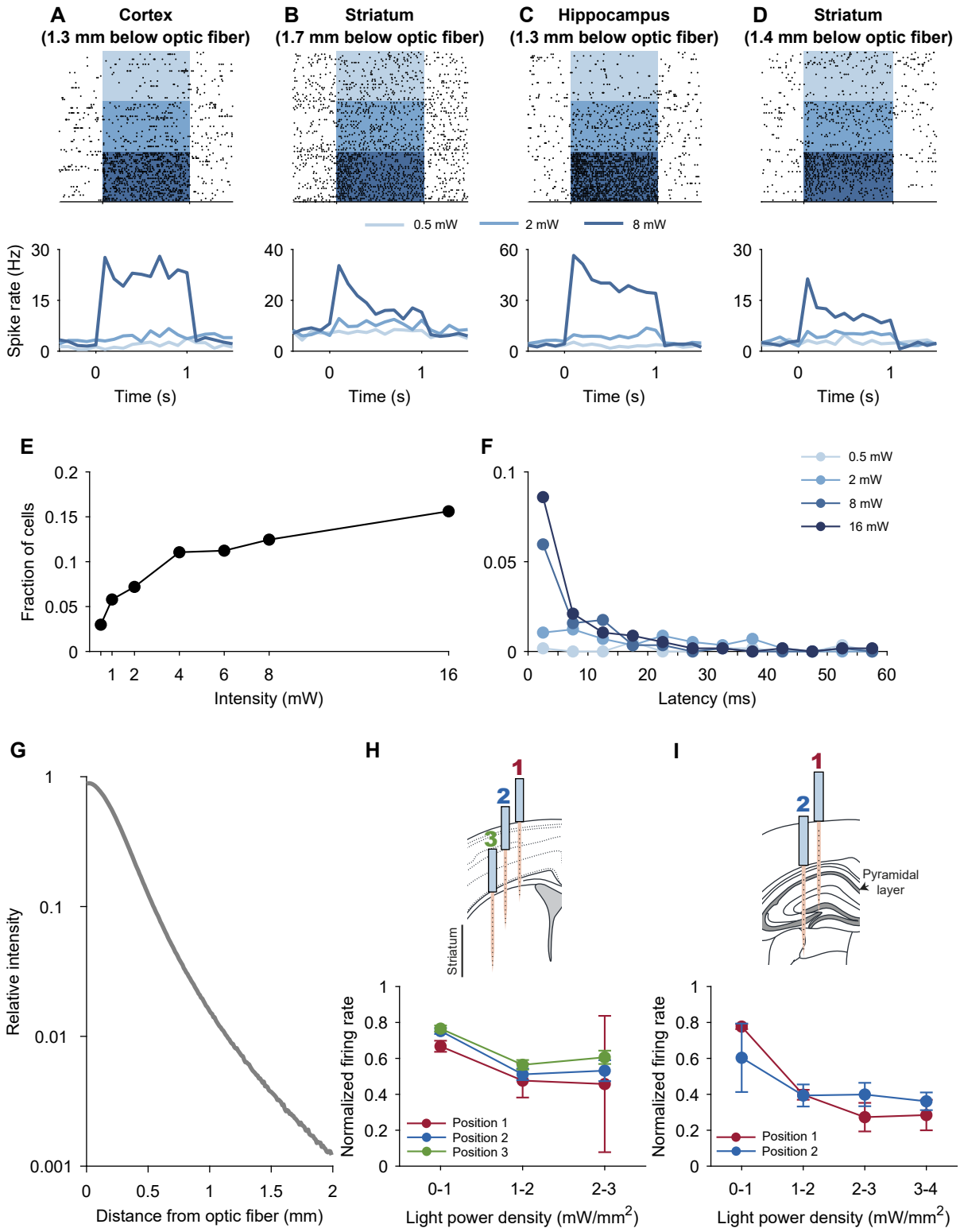

Figure S7, Optogenetic activation of interneurons far from the optic fiber and comparison of neuronal silencing at different optic fiber positions in VGAT-ChR2 mice, related to Figures 4 and 5. ***A-D***, Examples of neurons in cortex (***A***), striatum (***B***, ***D***) and hippocampus (***C***) that were recorded 1.3-1.7 mm from the optic fiber (distance is specified in heading). ***E-F***, Fraction of excited cells recorded >=1.2 mm from the optic fiber (***E***) and their response latency distribution (***F***). Tick marks on the x-axis of ***F*** represents the edges of 5-ms bins. ***G***, Estimated relative light intensity as a function of distance from the optic fiber (based on Stujenske et al., 2015; see STAR Methods). ***H***, Top, Inhibition in striatal neurons was compared when the optic fiber was above the cortex (Position 1), 0.5 mm below the cortical surface (Position 2) and above the striatum (Position 3). Bottom, Normalized firing rates as a function of light intensity when the optic fiber was in each of the three positions. ***I***, Top, Inhibition in CA1 pyramidal neurons was compared when the optic fiber was above the cortex (Position 1) or above the hippocampus (Position 2). Bottom, Normalized firing rates of CA1 pyramidal neurons as a function of light intensity when the optic fiber was in one of the two positions. Error bars represent the mean ± s.e.m. over neurons.

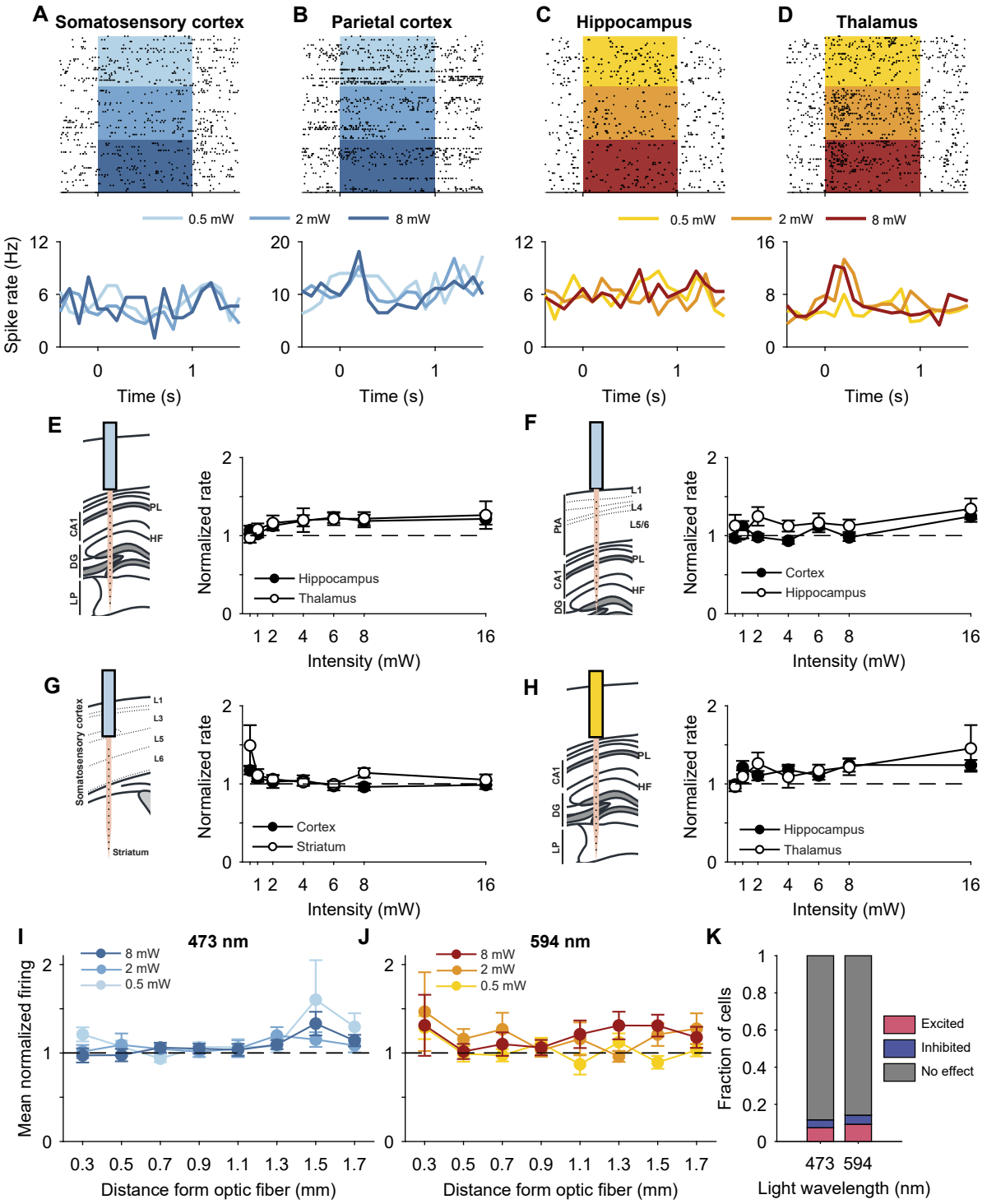

Figure S8, Effect of light on neuronal activity in mice without opsin expression, related to Figures 1-3 and 6. ***A-D***, Responses of neurons in the somatosensory cortex (***A***), parietal cortex (***B***), hippocampus (***C***) and thalamus (***D***) to blue (***A-B***) and yellow (***C-D***) light in opsin-free mice. Neurons in ***A-C*** were recorded close (<=0.5 mm) to the optic fiber. A few neurons showed transient excitatory responses (***B***, ***D***). ***E-G***, Normalized firing rates during blue light delivery to different brain regions: hippocampus and thalamus (***E***, compare with Figure 1), cortex and hippocampus (***F***, compare with Figure 2) and cortex and striatum (***G***, compare with Figure 3). ***H***, Normalized firing rates in hippocampus and thalamus during yellow light delivery (compare with Figure 6). ***I-J***, Normalized firing rates as a function of distance from the optic fiber for blue (***I***) and yellow (***J***) light stimulation. ***K***, Fraction of cells that were significantly excited by light stimulation at each wavelength. Error bars represent the mean ± s.e.m. over neurons.