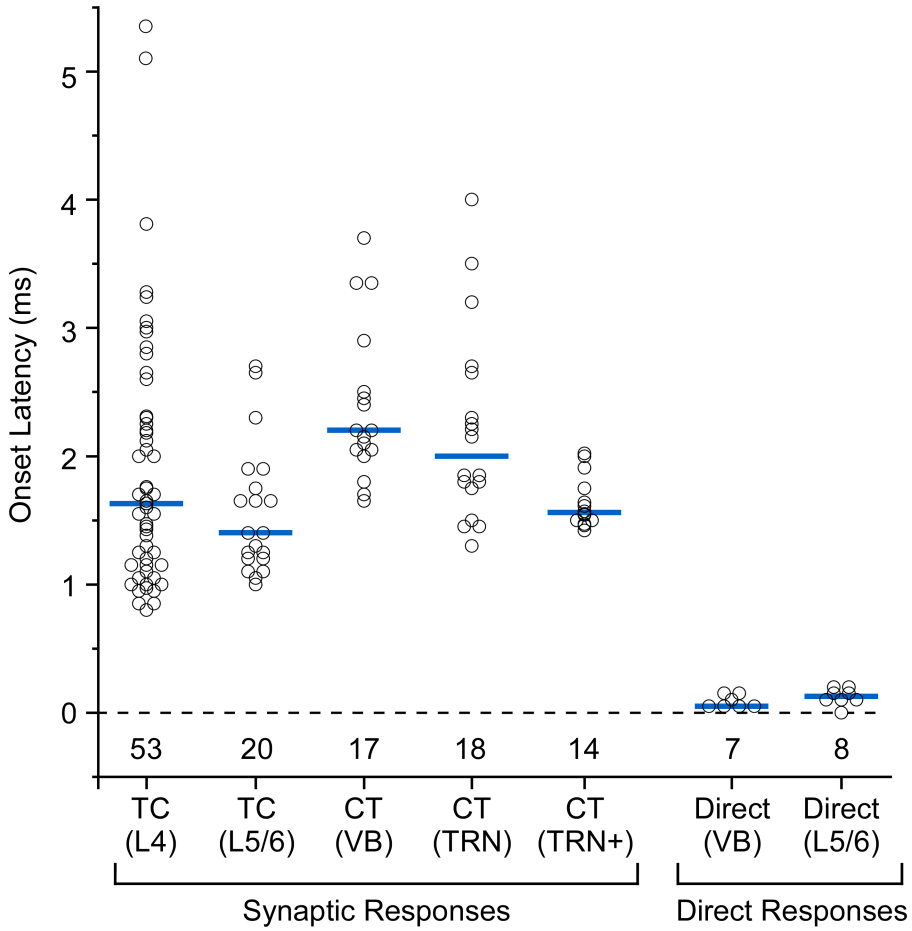
Inventory of Supplemental Information for Cruikshank et al,

(NEURON-D-09-00787R1)

- Our supplemental information includes 10 figures, with legends written below each.

- Supplementary Figure 1 is most closely associated with Main Figures 2, 3, 6, 7 and 8.
- Supplementary Figure 2 is most closely associated with Main Figures 2, 6, 7 and 8.
- Supplementary Figure 3 is most closely associated with Main Figure 3.
- Supplementary Figure 4 is most closely associated with Main Figures 3 and 4.
- Supplementary Figure 5 is most closely associated with Main Figure 6.
- Supplementary Figure 6 is most closely associated with Main Figure 6.
- Supplementary Figure 7 is most closely associated with Main Figures 4 and 8.
- Supplementary Figure 8 is most closely associated with Main Figure 8.
- Supplementary Figure 9 is most closely associated with Main Figures 8 and 9.
- Supplementary Figure 10 is most closely associated with Main Figures 8 & 9.

Supplemental Figure 1



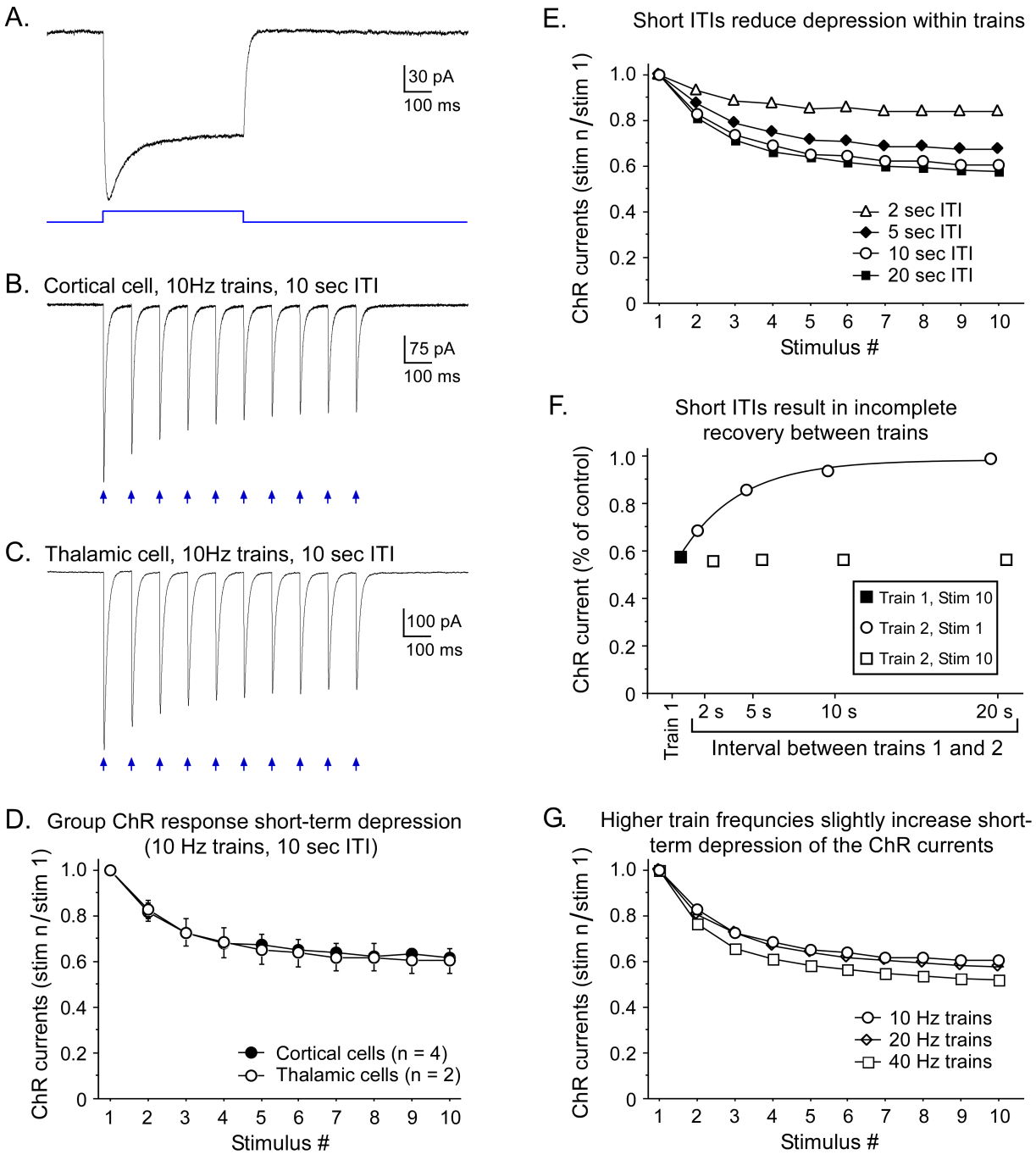
Supplementary Figure 1. Latencies of ChR2/laser-evoked responses in the thalamocortical and corticothalamic systems

Onset latencies for the thalamocortical (TC) and corticothalamic (CT) synaptic responses and for the direct responses in ChR2-expressing VB thalamic cells (VB) and cortical layer 5/6 cells (L5/6). The TC synaptic responses are divided according to the laminar location of the recorded cell and the CT synaptic responses are divided according to thalamic subdivision of the recorded cell (and according to injection parameters for TRN - explained below). Numbers below the zero line are group cell counts. Each circle represents the mean latency for one cell, measured with the highest laser intensity tested. Horizontal blue lines are median latencies for each group.

Onset latencies were significantly faster for direct than synaptic responses ($p < 0.0001$), with no overlap in latencies between the direct and synaptic categories.

There was a 4-5 ms range in latencies for synaptic responses. There were no significant differences in synaptic latencies between the pathways ($p > 0.34$, TC vs. CT), between layers within cortex ($p > 0.16$, TC-L4 vs. TC-L5/6) or between nuclei within thalamus when single virus injections were made in cortex ($p > 0.44$; CT-VB vs. CT-TRN). However, for one group of CT experiments in which TRN cell responses were recorded (CT-TRN+), we made 3 partially overlapping lentivirus injections into cortex (0.5 to 1.0 μl each), resulting in qualitatively stronger expression of ChR2/EYFP in the CT pathway compared with single injections. In those cases, the mean synaptic latencies were significantly faster than latencies following single injections ($p < 0.02$; CT-TRN+ vs. CT-TRN), although the fastest latencies in the two groups were quite similar.

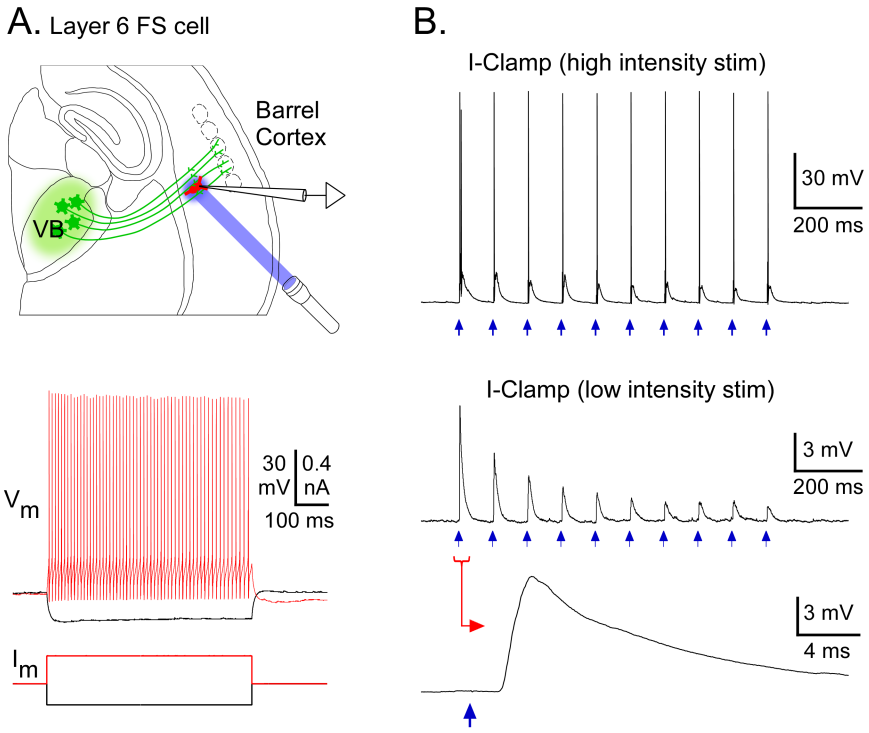
Supplemental Figure 2



Supplementary Figure 2. Dynamics of direct laser-evoked responses in ChR2-expressing cells

A. Application of a 500 ms laser pulse to a ChR2-expressing cortical cell evoked a transient peak inward current followed by a reduced amplitude sustained current as in Fig. 2C and previous reports (0.023 mW laser intensity; Vhold = -84 mV). **B.** 10 Hz trains of 1 ms laser pulses (0.11 mW) evoked inward currents that depressed within the trains (average of 10 sweeps, 10 s inter-train-intervals (ITIs)). **C.** Response depression in a ChR2-expressing thalamic cell to the same train frequency (3 mW, 0.05 ms pulses). **D.** The depression pattern was similar for cortical and thalamic cells, with steady-state responses at about 62% of control. The 10 Hz, 10 sec ITI trains quantified here were the same as the rates used for synaptic stimulation throughout this paper. Comparable depression of ChR2 currents likely occurred in presynaptic terminals and arbors of the TC and CT axons. **E.** Shortening the time between trains attenuated depression within trains. **F.** There was incomplete recovery with short ITIs. For this plot, pairs of 10 Hz trains were delivered at several ITIs. Evoked currents were normalized to the response to the first stimulus of the first train in each pair. By the 10th pulse of the first train, responses were depressed to 58% of control (filled square). The response remained strongly depressed at the beginning of the second train when the interval between trains was 2 seconds (open circle, 2 s) and there was little further depression through the 10th stimulus in that train (open square at 2 s). Longer ITIs resulted in progressively greater recovery and resulted in more depression within the second train. The curved line is an exponential fit to estimate the time constant of recovery from depression/desensitization (~ 4 sec.). The failure to recover between trains largely accounts for the attenuated depression observed in E; the ChR2 was already partly desensitized during the initial stimuli within trains 2-10, so subsequent intra-train decreases to the fully desensitized state were less pronounced than if started from a recovered state. **G.** There is only a small change in depression with increased train frequencies (20-40 Hz). For this panel the ITI was 10 sec. The data in E-G were averages from two ChR2-expressing thalamic cells.

Supplemental Figure 3

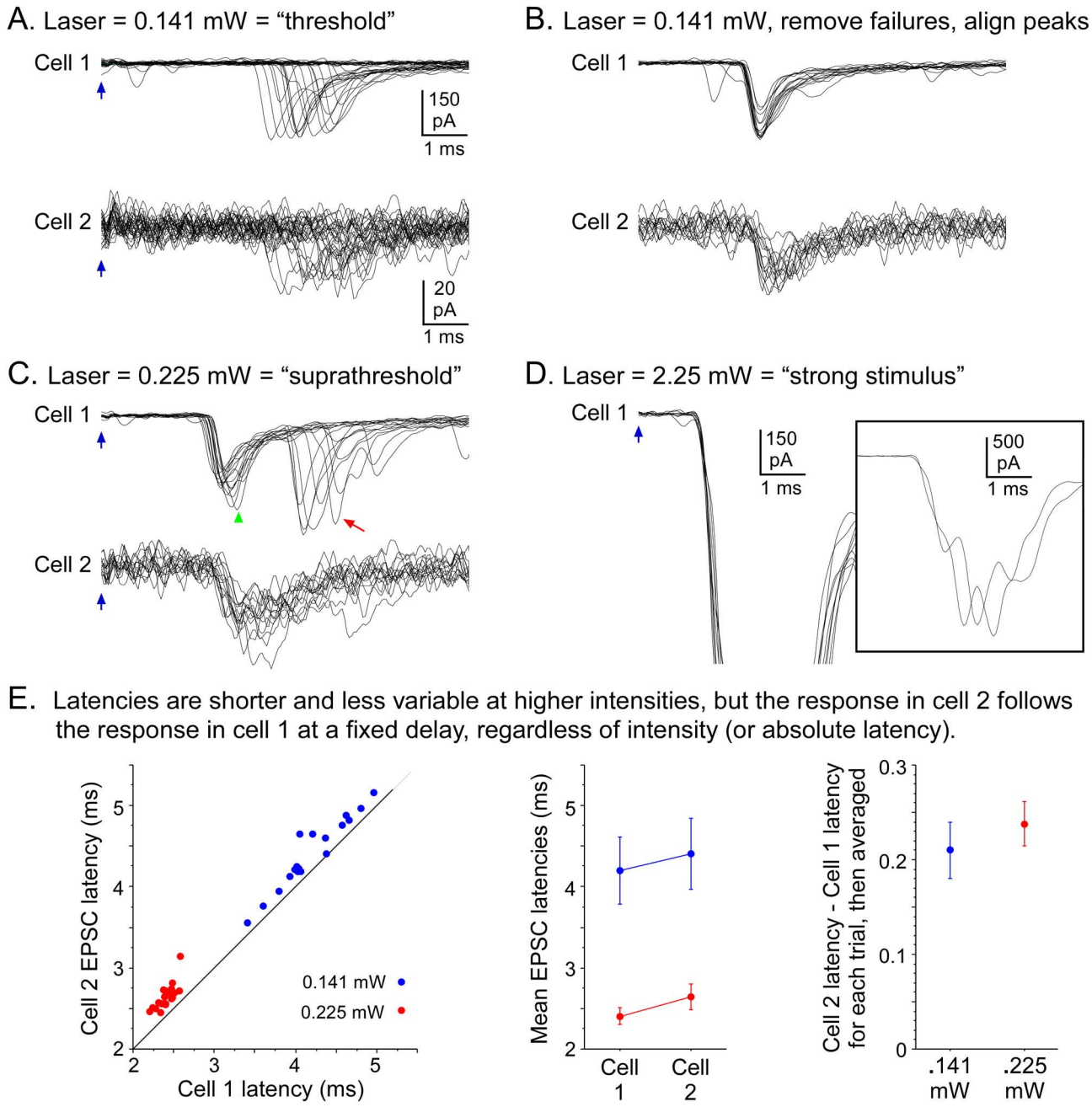


Supplementary Figure 3. Laser stimulation of ChR2-expressing thalamocortical arbors in cortex evoked strong synaptic excitation in non-expressing cortical neurons of layer 6

A. A(top): Cartoon of experimental configuration, indicating ChR2 expression in VB cells and their TC axons (green), and recording in non-expressing FS cell in cortical layer 6 (red). Laser stimuli (blue) were directed at TC arbors surrounding the recorded cell. A(bottom): Responses of the FS cell to intracellular current steps.

B. TC synaptic responses to 10 Hz trains of laser flashes (blue arrows). B(top): Action potentials were evoked on each flash for high intensity stimuli (1.0 ms duration, 4.5 mW). B(middle): When flash durations and intensities were reduced (0.1 ms, ~ 1.1 mW), subthreshold EPSPs were evoked, depressing across the train. B(bottom): Expanded trace shows ~ 1.8 ms synaptic delay. Baseline membrane potentials were ~ -79 mV.

Supplemental Figure 4



Supplementary Figure 4. Changes in thalamocortical EPSC latencies as laser intensities are increased from threshold

A. Laser stimulation over a pair of simultaneously recorded layer 6 cells at threshold intensity for TC responses (cell 1 was an FS, cell 2 an RS; 0.1 ms pulses, 1/10 sec). Stimuli were at the start of each sweep. 30 consecutive sweeps shown; response failures occurred on the same 15 trials for cells 1 and 2. Notice the relatively long and variable response latencies.

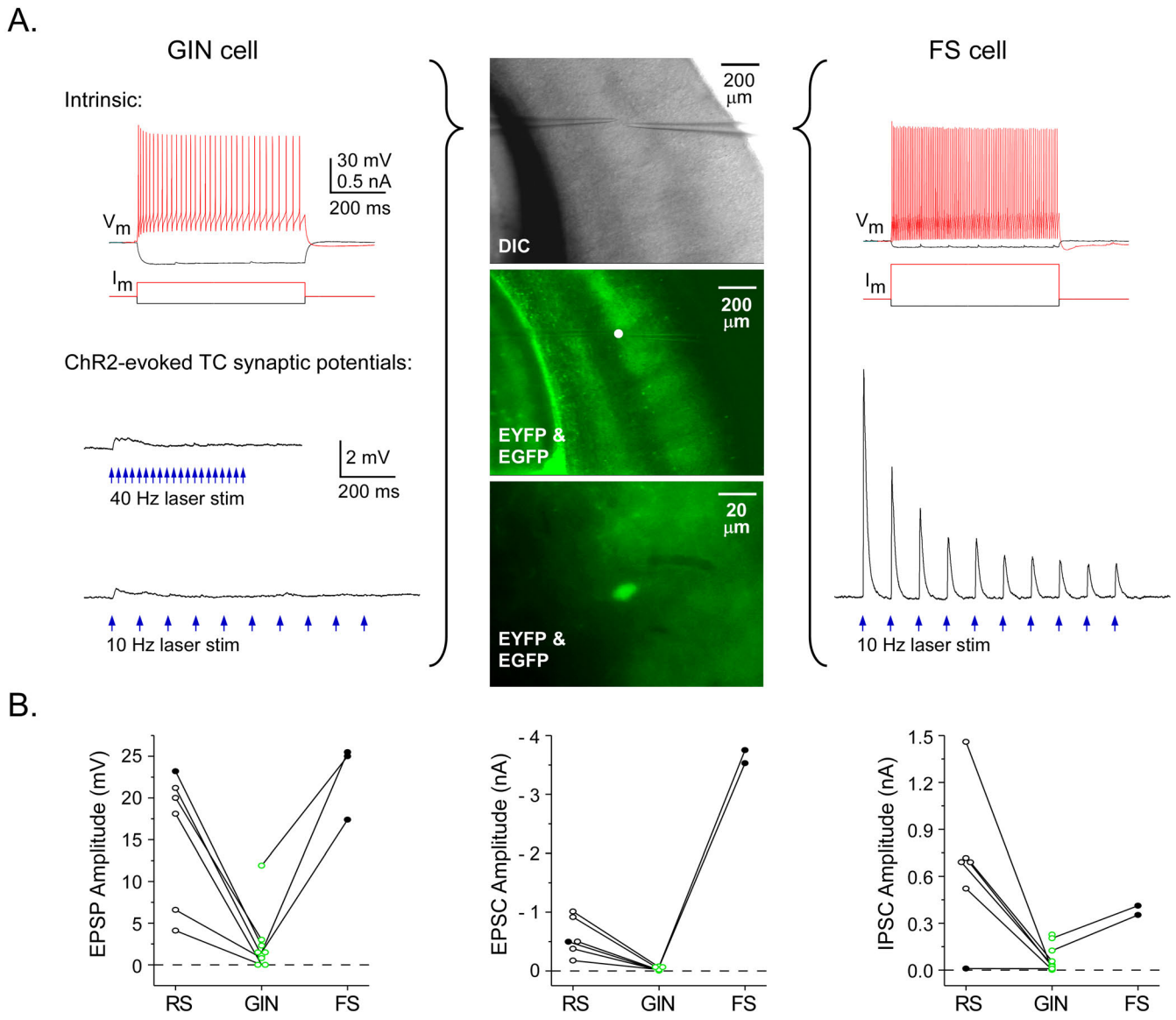
B. To visualize response shapes, failures were removed and traces were aligned to the peaks of the cell 1 EPSCs. Amplitudes and shapes were fairly constant, although the signal-to-noise ratio in cell 2 was poor. Notice that responses in cell 2 are aligned with those in cell 1 (with a slight delay - see panel E below).

C. Slightly stronger laser intensities (0.225 mW) produced more reliable responses; there were no failures (15 sweeps shown) and latencies were shorter and less variable (compare to panel A). The initial events evoked by 0.225 mW were similar to those at threshold (aside from latency - compare to panel B). However, additional events were evoked in cell 1 just after the first peak (green arrowhead) or about 2 ms later (red arrow), consistent with the suprathreshold stimulation causing action potentials at additional release sites.

D. 10-fold increases in laser intensity increased EPSC amplitudes and decreased latencies and jitter. However, large responses were generally multi-peaked (inset).

E. The left panel compares EPSP onset latencies of cells 1 and 2 for individual trials (onset latency defined as time at 20% of peak). Latencies were longer and more variable at the lower intensity. However the difference between cells 1 and 2 were fairly constant and independent of intensity or absolute latency. These observations are quantified in the middle and right panels. Error bars indicate standard deviation in this figure. The relatively constant delay between responses in cells 1 and 2 (combined with failures on common trials) despite wide variation in absolute latencies suggests that the near-threshold synaptic responses in these two cells were dependent on a common presynaptic axon. Presumably action potentials were initiated nearer the release site on cell 1 than cell 2, then subsequently propagated to cell 2. Thus, release onto cell 2 was likely to be mediated by a normal axon spike-initiated event and not by directly exciting its presynaptic bouton.

Supplemental Figure 5



Supplementary Figure 5. "GIN" type interneurons had weaker thalamocortical responses than FS or RS cells

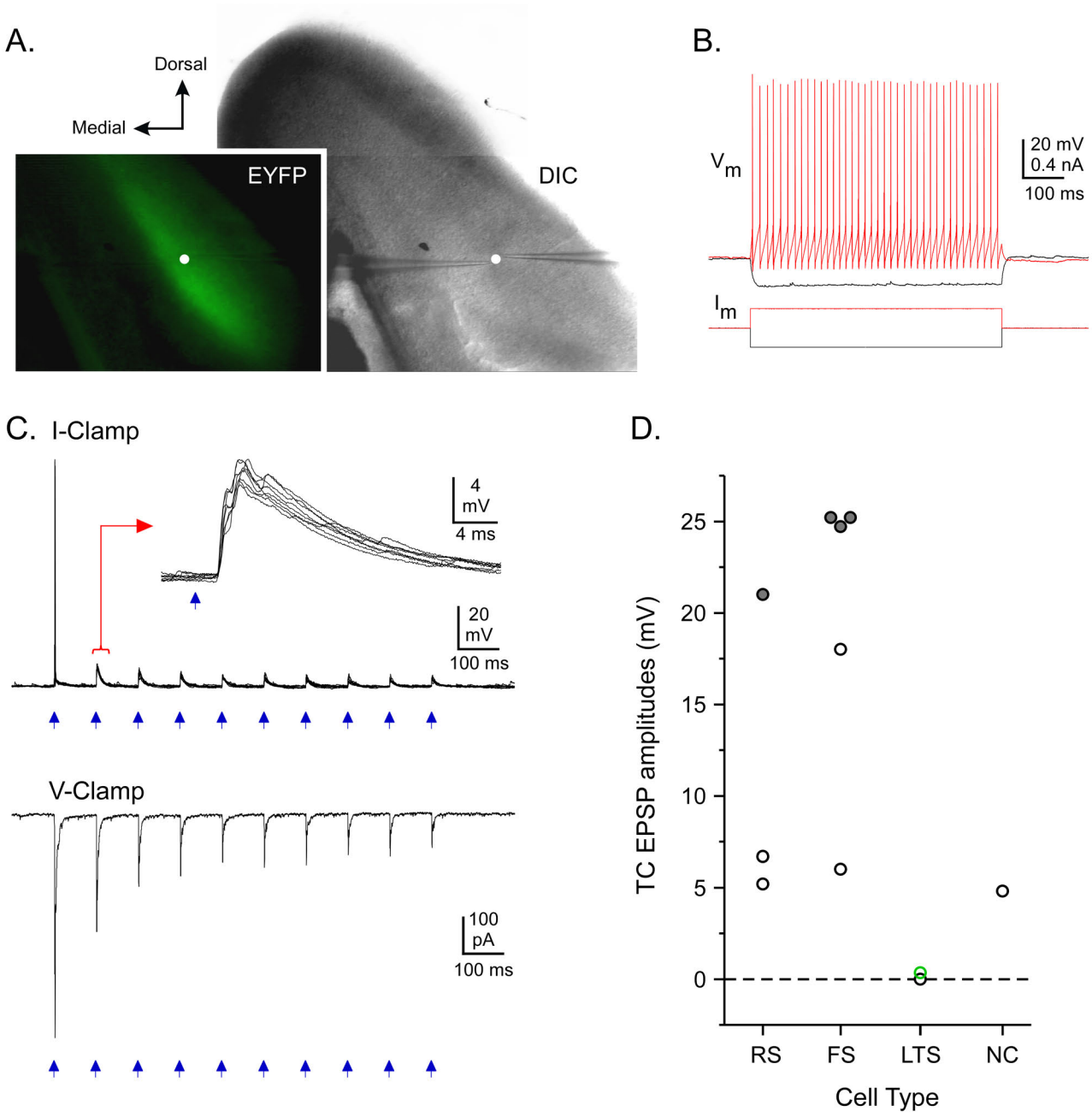
A, Middle: DIC and fluorescence images of a live slice from a GIN mouse (EYFP filters used). The small white circle in the center panel ("EYFP & EGFP") indicates the position of the recorded cells and laser stimuli in lower layer 4. VB thalamus had been injected with virus and TC cells and their cortical axons expressed ChR2/EYFP fusion protein. Small bright dots in layers 3/4 are EGFP-expressing GIN somata, visible among the EYFP/ChR2-expressing TC axons. Lower panel shows an EGFP-expressing GIN cell at high magnification; we subsequently recorded from this cell (left).

A, Left: Recordings from a GIN cell. Top: intrinsic responses to current injections. The physiological characteristics of the GIN cells matched our LTS category, consistent with previous observations (Fanselow et al., 2008). Bottom: synaptic potentials evoked by laser stimulation of TC arbors (0.1 ms, 4.5 mW). Both 10 Hz and 40 Hz trains evoked weak depressing EPSPs (<1 mV from -80 mV V_m).

A, Right: Traces from a simultaneously recorded FS cell located ~ 30 μm from the GIN cell. The TC synaptic potentials of this FS cell were much larger than those of the GIN cell (using matching steady-state V_m; laser aimed between the 2 cells). Longer duration laser stimuli (1 ms) caused > 25 mV EPSPs that triggered spikes in this FS cell while evoking ~1.5 mV EPSPs in the GIN cell (see B, left).

B. Group data comparing EPSPs (left), EPSCs (middle) and feedforward IPSCs (right) evoked by ChR2 TC stimuli in GIN-RS and GIN-FS cell pairs. EPSPs were recorded in current clamp from V_m of -74 to -84 mV. EPSCs measured in voltage clamp from -84 to -89 mV (near the inhibitory reversal potential). IPSCs were measured as the peak outward current when $V_{command}$ was between -49 and -54 mV. Diagonal lines connect responses of simultaneously recorded cell pairs (< 40 μm separation). Filled symbols indicate cells for which TC stimuli triggered action potentials when in current clamp. n = 6 RS-GIN and n = 3 FS-GIN pairs (1 FS cell was lost before voltage clamp recording).

Supplemental Figure 6



Supplementary Figure 6. ChR2/laser-evoked thalamocortical responses in the visual cortex

A. Fluorescent and DIC images of the recording location in layer 4 of visual cortex (images are aligned horizontally). The virus injection site included the lateral geniculate nucleus (not shown). Bright fluorescence from EYFP/ChR2-expressing thalamocortical arbors can be seen in layer 4 of the visual cortical area. The location of the cell and the laser target is indicated by the white dots. Recordings in B-D are from the lateral (right) electrode.

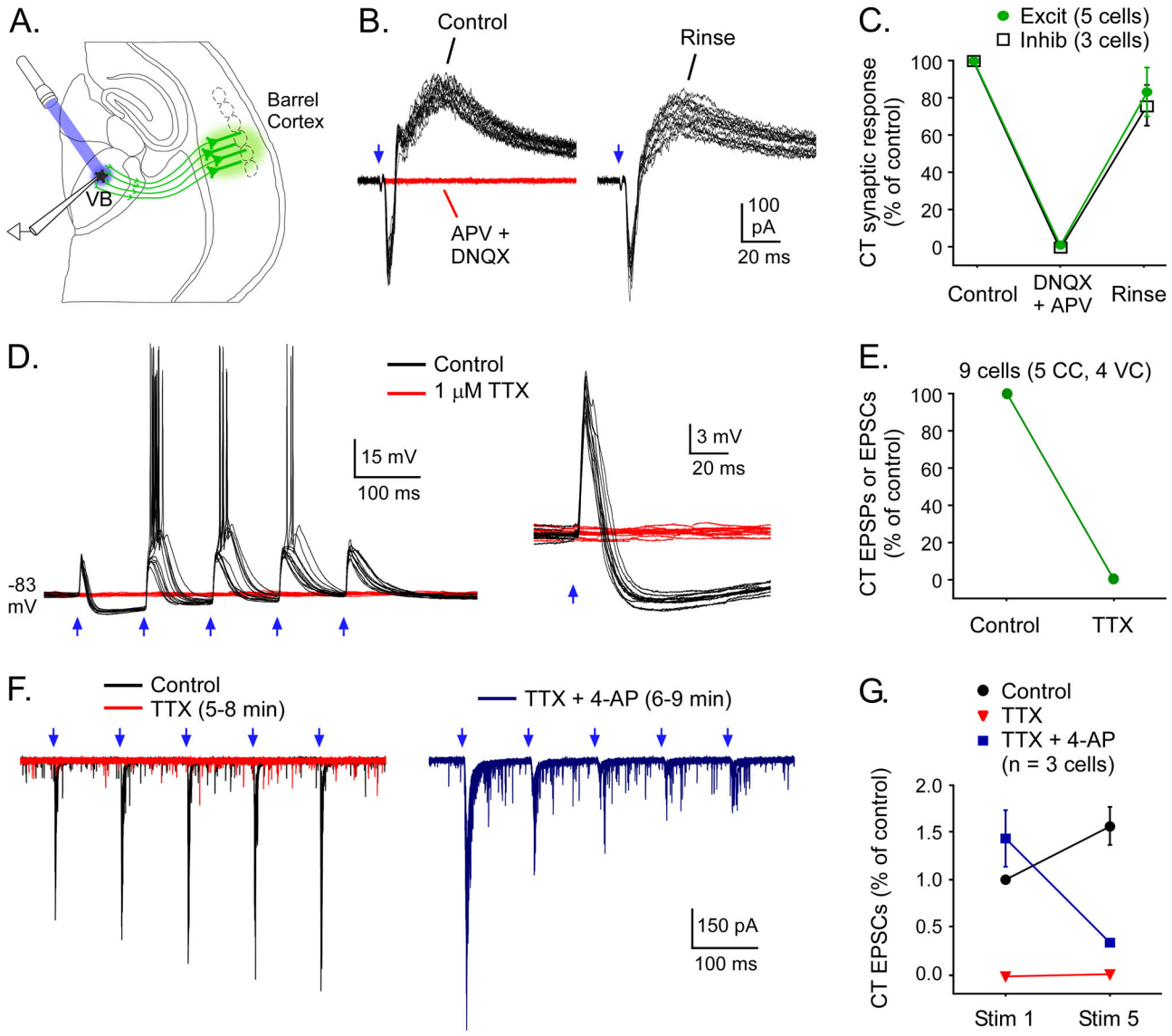
B. Responses to intracellular current steps, indicating a fast-spiking (FS) cell type.

C top. Current-clamp responses to trains of 1 ms laser flashes (10 Hz, 10 sec inter-train-intervals; 8 superimposed sweeps). Action potentials were evoked on the first flash in each train, then progressively depressing EPSPs were evoked subsequently. The inset shows responses to the second stimulus of the trains.

C bottom. Large synaptic currents were evoked by the same trains of laser stimuli (average of 9 sweeps, -84 mV holding potential). Responses depressed throughout the train.

D. TC-evoked EPSP amplitudes in visual cortical layer 4 were strongest in FS cells and weakest in LTS cells (11 neurons from 4 mouse brains). Cell-type abbreviations and conventions similar to Figure 6. Laser flashes were 1 ms, 4.5 mW. All excitatory responses depressed with 10 Hz stimulation. There were also clear evoked inhibitory responses in 10/10 tested cells (not shown), including the LTS cell that lacked excitation. The non-classified neuron was not tested for inhibition. Green symbol indicates EGFP-positive GIN cell.

Supplemental Figure 7



Supplementary Figure 7. ChR2/laser-evoked corticothalamic responses require glutamate receptors and action potentials under control conditions

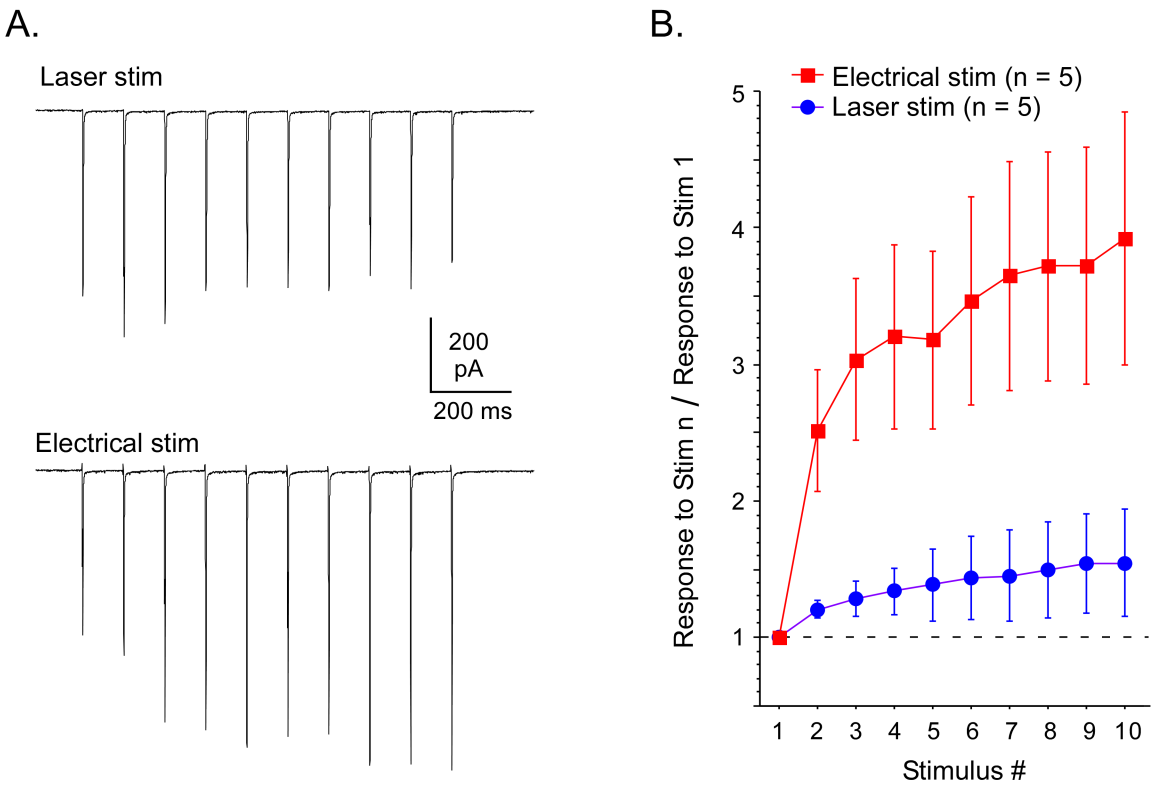
A. Experimental configuration: ChR2-expressing CT projections (green) and a non-expressing thalamic relay cell (black). Laser stimuli were directed at the CT arbors surrounding the recorded thalamic cell. Group data that follow include both VB and TRN cells.

B. In a VB cell voltage clamped at -72 mV, CT stimuli (arrows) evoked inward EPSCs and outward IPSCs ("Control", black traces). APV (50 μM) and DNQX (20 μM) for 6 min blocked both PSC types (red traces). PSCs recovered after 55 min in control ACSF ("Rinse"). 15 sweeps shown for each period. **C.** Group effects of DNQX+APV on CT responses. Error bars = SEM. For each cell at each period, 9-15 sweeps (3-5 min) were averaged.

D. In a VB cell recorded in current-clamp, 10 Hz trains of CT stimuli evoked an EPSP/IPSP sequence on the first pulse in each train; the next 3 stimuli often evoked spikes (left panel, black). TTX perfusion for ~ 5 min blocked responses completely (red). Responses to the 1st pulse are magnified on right (12 trials per condition shown). **E.** Group effects of TTX. CT-evoked EPSPs/EPSCs were blocked within 5-12 min and did not recover during 20-30 min rinses. Effects of TTX on inhibition in the CT pathway were not systematically tested.

F-G. Laser stimulation of ChR2-expressing CT axons in the presence of TTX (1 μM) and 4-AP (1 mM). **F.** In a TRN cell, voltage clamped at -79 mV in control ACSF, a 10 Hz stimulus train evoked facilitating EPSCs ("Control" - black traces). After blocking responses with TTX (red traces), 4-AP was added (blue traces, right). This "rescued" the CT responses, although responses to 1st stimuli in trains increased in amplitude and repetitive stimuli led to depression rather than facilitation. 10 sweeps are shown per period. **G.** Group CT data comparing 1st and 5th EPSC amplitudes evoked by 10 Hz stimuli. EPSCs are normalized to response in control condition, 1st stimulus. EPSC latencies and durations also increased in 4-AP + TTX compared to control (not shown).

Supplemental Figure 8

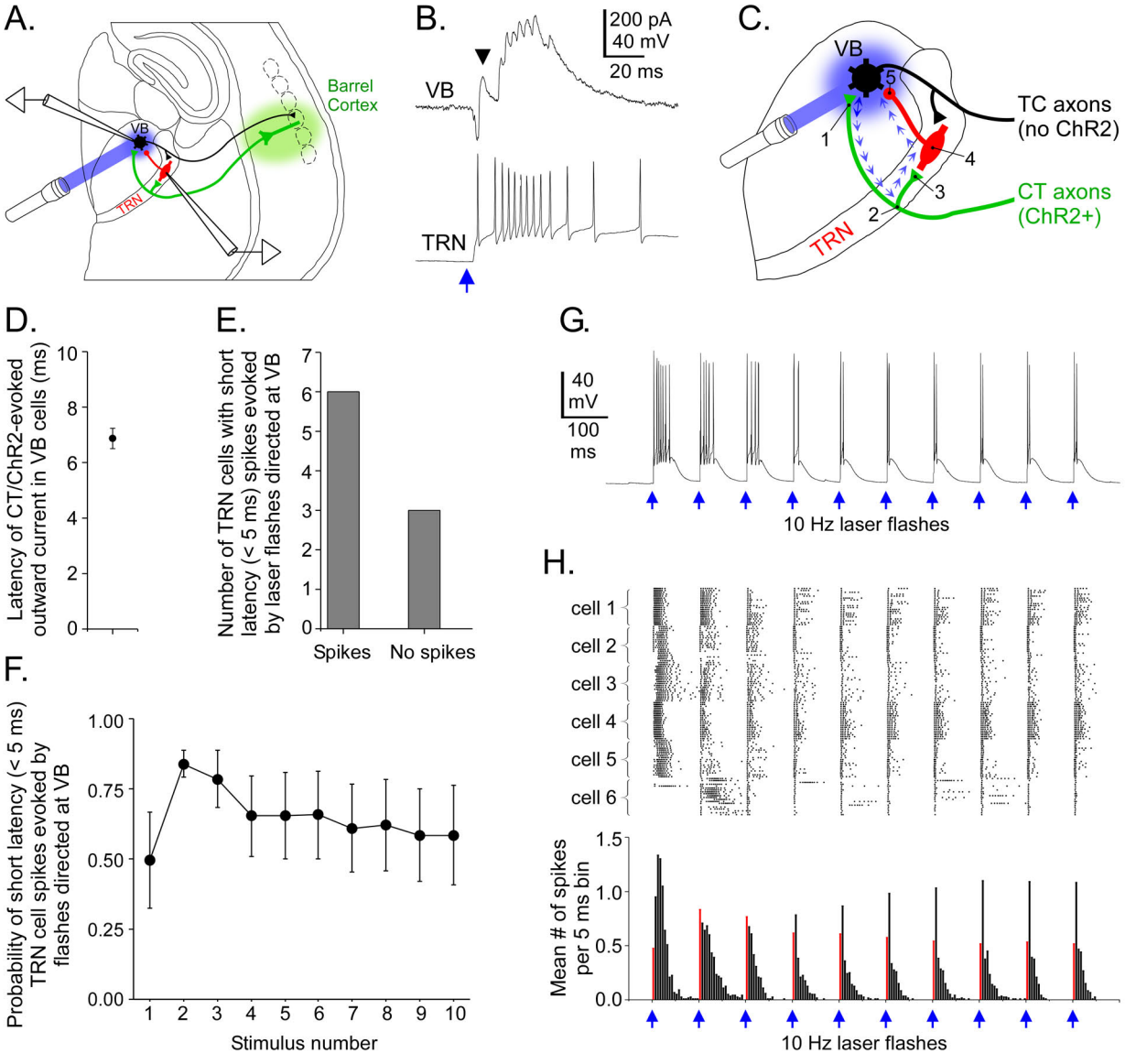


Supplementary Figure 8. Direct comparison of the response dynamics produced by ChR2/laser-evoked corticothalamic stimulation and electrical stimulation of the internal capsule

A. Comparison between responses of a TRN cell to ChR2/laser stimulation of CT afferents and electrical stimulation of the internal capsule. Laser stimuli (1.13 mW, 0.2 ms) were delivered directly over the TRN cell as usual. Electrical stimuli (0.2 ms pulses, 22 μA) were delivered via a bipolar stimulating electrode (Methods). Both types of stimuli were delivered at 10 Hz. Traces are averages of 30-50 sweeps. For this cell, responses to laser stimuli moderately facilitated during the first few pulses in a train, then depressed slightly on later stimuli. In contrast, responses to electrical stimuli facilitated throughout the train. The holding potential was -84 mV.

B. Group data comparing CT responses to laser and electrical stimuli for 5 cells (3 VB and 2 TRN). Facilitation was significantly greater for electrical than laser stimuli ($p < 0.0001$, paired t-test). In fact for all 5 cells, short–term plasticity ratios (response n / response 1) were higher for electrical than laser stimulation for pulses 3 through 10 (not shown).

Supplemental Figure 9



Supplementary Figure 9. Evoked inhibition in VB is likely to be mediated through corticothalamic activation of TRN cells

A. Diagram of recording configuration with simplified schematic of TC/CT circuitry. ChR2 expression is in CT cells and axons (green). Recordings in non-expressing VB relay (black) and/or TRN (red) cells. Laser (blue) directed at CT arbors near VB cell.

B. Simultaneous recording of ChR2/laser-evoked CT responses from TRN (I-clamp) and VB (V-clamp) cells. The TRN cell (bottom) had an initial fast spike, a brief pause, and finally a burst of spikes. The VB cell had an initial inward EPSC, a fast outward IPSC (arrowhead), then a pause, and finally burst of outward IPSCs. These 2 cells were not synaptically coupled, but the correlated temporal patterns of the TRN spikes and VB IPSCs suggests that TRN cells with similar spike patterns evoked the VB IPSCs.

C. Simplified diagram of the circuitry that likely mediates feedforward inhibition in VB. ChR2-expressing CT axons near the recorded VB cell are excited by blue laser (1). Spikes propagate along the CT axons in two directions (arrows indicate directions of spike propagation); they travel orthodromically, leading to release of glutamate from the CT terminals (and fast EPSCs in VB cells) and they also travel antidromically toward cortex. The antidromic spikes would invade collaterals to TRN (2) which would then propagate normally to the CT terminals within TRN (3) causing glutamate release and excitation of TRN cells. Spike bursts would be evoked in TRN cells (4) ultimately leading to GABA release from TRN neuron terminals (5) and IPSCs in VB cells.

D. The mean latency (± SEM) of outward inhibitory currents evoked in VB cells by laser CT stimulation was 6.87 ± 0.37 ms.

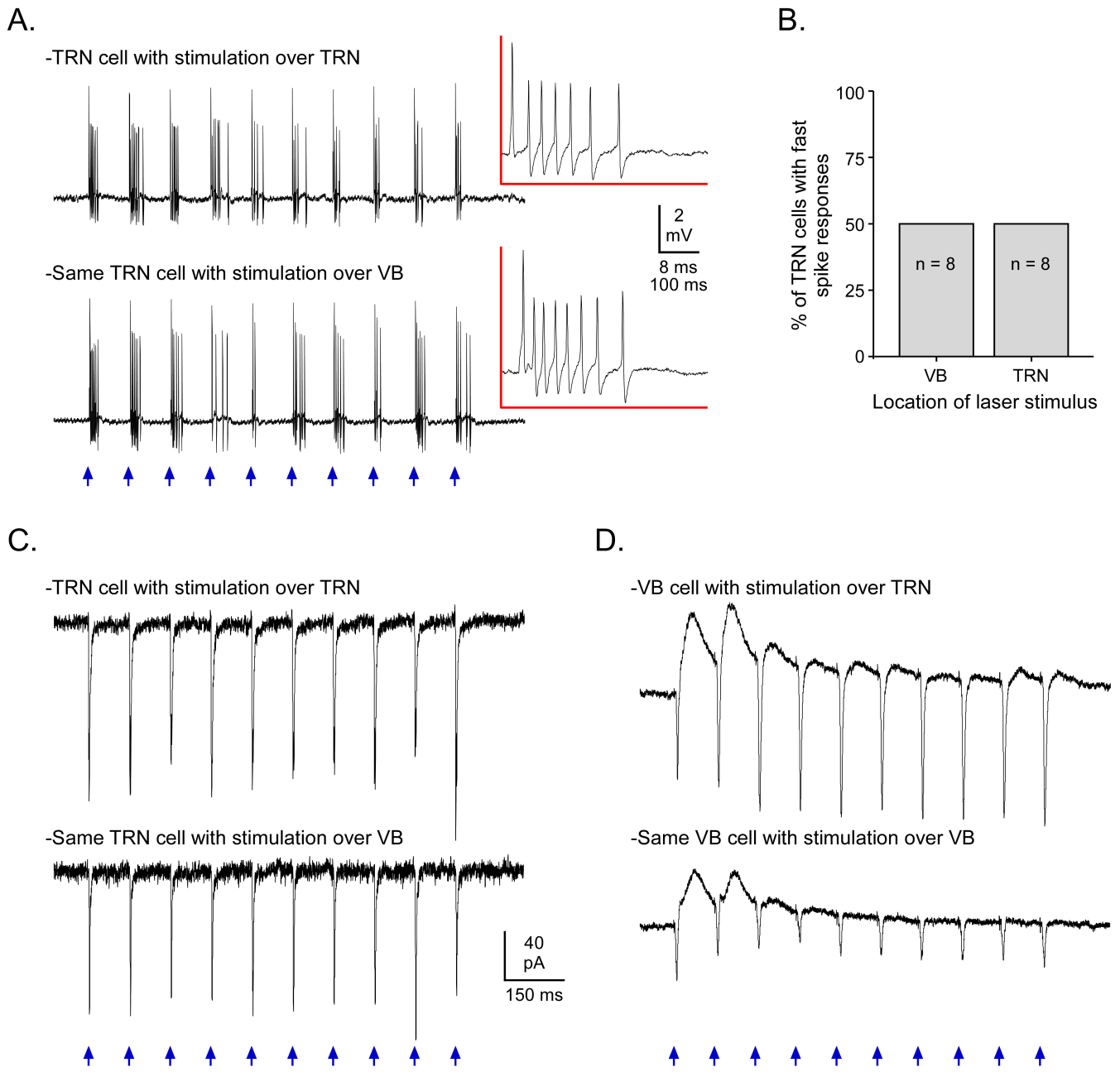
E. TRN cells were recorded while laser-stimulating CT axons within VB, to determine if TRN cells fired APs with latencies that could account for the IPSCs typically recorded in VB cells. The VB laser stimuli and TRN cells were located among strongly labeled bundles of ChR2/EYFP expressing CT arbors. Of the 9 TRN cells tested, 6 exhibited consistent (> 0.5 probability) spikes with short (< 5 ms) latencies for one of the first 2 pulses in 10 Hz trains, consistent with observed IPSCs in VB (panel D above and Figure 9).

F. Probabilities of observing APs in TRN cells within 5 ms of the VB laser stimuli (group of 6 TRN cells). Probabilities were highest for 2nd stimulus in 10 Hz trains, which corresponds to the stimulus causing strongest inhibition in VB cells (Figure 9).

G. Responses to a single laser train for a TRN cell. Laser applied to VB (blue arrows show stimulus times).

H. Peristimulus spike rasters (top) and group histogram (bottom) for 6 TRN cells exhibiting short latency spikes following VB laser stimulation (1 ms flashes). Each row in the raster plot corresponds with a single train (20 rows per TRN cell). Dots indicate spike times. Histogram shows number of spikes per 5 ms bin, averaged across 20 sweeps per cell for all 6 cells. Red bars correspond to the 1st 5 ms bin after onset of the laser stimulus.

Supplemental Figure 10



Supplementary Figure 10. Failure to observe corticothalamic inhibition in TRN cells is not due to weak excitation of TRN or to VB being a necessary stimulation locus for eliciting corticothalamic inhibition

A. Example of a TRN cell recorded in cell-attached configuration; 10 Hz laser stimulation of ChR2-expressing CT axons applied either to the TRN itself (top) or to the VB (bottom) evoked strong synaptic excitation and bursts of action potentials (blue arrows indicate laser flashes). Insets show responses to 1st stimuli in the trains; the beginnings of the inset traces correspond to stimulus onset times. The latency to evoke spikes was slightly longer when stimuli were applied to VB, consistent with the longer conduction distance.

B. Laser stimulation of VB or TRN triggered CT-evoked spike responses in 4 of 8 TRN cells for which both stimulus locations were tested. Spikes had consistently short latencies (< 5 ms) in these 4 cells. The remaining 4 cells did not respond with short latency spikes to stimulation of either nucleus, although they had clear subthreshold excitatory synaptic responses. All slices used for this comparison contained strong CT axonal labeling within TRN and VB.

C. Responses of a TRN cell recorded in voltage clamp to laser stimulation of CT axons in the TRN (top) and in the VB (bottom). Neither stimulation locus evoked any inhibitory (outward) currents in the TRN, but both loci evoked clear excitatory (inward) currents. The holding potential was -54 mV.

D. Responses of a VB cell to laser stimulation of CT axons in the TRN (top) and in the VB (bottom). Stimulation at either locus evoked clear excitation and inhibition in this VB cell. The holding potential was -74 mV.

Overall, these data indicate that (1) TRN cells are driven well when stimuli are applied to the TRN and that (2) laser stimulation of the VB is neither necessary nor sufficient to evoke CT feedforward inhibition. Group data supporting this conclusion are provided in the main Results section. Thus, the data suggest that the exact placement of stimuli along the CT afferents is not critical for triggering inhibition in VB cells or for the absence of CT-evoked inhibition in TRN.