Optogenetic Stimulation of the Corticothalamic Pathway Affects Relay Cells and GABAergic Neurons Differently in the Mouse Visual Thalamus

Chris W. D. Jurgens, Karen A. Bell, A. Rory McQuiston, William Guido*

Department of Anatomy and Neurobiology, Virginia Commonwealth University Medical Center, Richmond, Virginia, United States of America

Abstract

The dorsal lateral geniculate nucleus (dLGN) serves as the primary conduit of retinal information to visual cortex. In addition to retinal input, dLGN receives a large feedback projection from layer VI of visual cortex. Such input modulates thalamic signal transmission in different ways that range from gain control to synchronizing network activity in a stimulus-specific manner. However, the mechanisms underlying such modulation have been difficult to study, in part because of the complex circuitry and diverse cell types this pathway innervates. To address this and overcome some of the technical limitations inherent in studying the corticothalamic (CT) pathway, we adopted a slice preparation in which we were able to stimulate CT terminal arbors in the visual thalamus of the mouse with blue light by using an adeno-associated virus to express the light-gated ion channel, ChIEF, in layer VI neurons. To examine the postsynaptic responses evoked by repetitive CT stimulation, we recorded from identified relay cells in dLGN, as well as GFP expressing GABAergic neurons in the thalamic reticular nucleus (TRN) and intrinsic interneurons of dLGN. Relay neurons exhibited large glutamatergic responses that continued to increase in amplitude with each successive stimulus pulse. While excitatory responses were apparent at postnatal day 10, the strong facilitation noted in adult was not observed until postnatal day 21. GABAergic neurons in TRN exhibited large initial excitatory responses that quickly plateaued during repetitive stimulation, indicating that the degree of facilitation was much larger for relay cells than for TRN neurons. The responses of intrinsic interneurons were smaller and took the form of a slow depolarization. These differences in the pattern of excitation for different thalamic cell types should help provide a framework for understanding how CT feedback alters the activity of visual thalamic circuitry during sensory processing as well as different behavioral or pathophysiological states.

Introduction

The dorsal lateral geniculate nucleus (dLGN) of the thalamus is the principal relay of retinal information to the visual cortex. However, the vast majority of synapses in this nucleus arise from nonretinal sources and provide a powerful substrate for modulating retinogeniculate signal transmission [1, 2] (see Fig. 1). Perhaps the largest source of nonretinal input to the dLGN is the feedback projection from layer VI cells of the visual cortex [3–5]. Corticothalamic (CT) input to the dLGN has been shown to regulate tonic and burst firing modes [6, 7], contribute to the receptive field structure of dLGN neurons [8–10] and synchronize activity between the thalamus and cortex in a stimulus-specific manner [11, 12]. Nonetheless, the cellular mechanisms underlying these effects remain unresolved, in part because the CT pathway innervates a number of different thalamic cell types that are also connected to each other (Fig. 1). In addition to providing direct excitatory input to dLGN relay cells [5, 13], CT afferents send collaterals to the visual sector of the adjacent thalamic reticular nucleus (TRN) [14, 15], which contain GABAergic neurons that make feedback connections with dLGN cells [16–18]. CT inputs to the dLGN also innervate GABAergic intrinsic interneurons [4, 19, 20] that synapse directly onto relay cells [5, 21]. Thus the CT pathway, while providing direct excitation to these three cell types, can also activate convergent inhibitory input onto relay cells.

While the slice preparation has proven to be a valuable assay, the reliance on electrical stimulation to untangle the interactions between CT neurons and different thalamic cell types has been challenging, and in some cases has produced variable results [22–27]. Besides the obvious difficulty in preserving CT connections in a slice preparation, the placement of stimulating electrodes in adjacent thalamic nuclei to stimulate CT axons can lead to the inadvertent activation of other thalamic circuits, such as those between the dLGN and the TRN [20, 29] or even those that involve ascending cholinergic projections arising from the brainstem [30, 31]. These problems are further exacerbated when attempting to study the effects of CT stimulation in the developing thalamus, especially at postnatal ages when CT projections are beginning to innervate the dorsal thalamus [32]. To overcome these technical obstacles, and to gain further insight as to how CT...
optogenetic approach. Input affects the activity of different thalamic cell types, we took an
dLGN (bottom), TRN (middle) and cortex (top).

to examine and compare CT-evoked synaptic responses in relay

Methods

Animals

All breeding and experimental procedures were approved by
the Virginia Commonwealth University Institutional Animal Care
and Use Committee (protocol AD20205). Experiments were
carried out using three different transgenic strains in which GFP
was used to visualize either layer VI neurons in the neocortex
(golli-t-GFP) [32], GABAergic neurons in the TRN (GAD65-
GFP) or intrinsic interneurons in the dLGN (GAD67-GFP; JAX
stock #007677). In the golli-t-GFP mouse, the golli promoter
drives the expression of a tau-enhanced green fluorescent protein
(t-GFP) fusion protein in layer VI neurons including their
descending projections throughout thalamus. In the GAD65-
GFP mouse, the GAD65 promoter drives the expression of GFP in
GABAergic neurons in the TRN, but not the dLGN. In the
GAD67-GFP mouse, the GAD67 promoter drives the expression
of GFP in intrinsic GABAergic interneurons in the dLGN, but not
the TRN. All founder lines were on a pigmented background (i.e.,
CB6F1/J, B6;129SV or C57/BL6). In total, experiments were
performed on 57 mice (19 golli-t-GFP, 15 GAD65-GFP and 23
GAD67-GFP).

Virus Injections

To photoactivate CT terminal arbors in the dLGN or the TRN,
an AAV carrying a vector for ChIEF fused to tdTomato was
injected into layer VI of the visual cortex. To make this vector,
cDNA from a mammalian codon optimized clone of ChIEF, fused
to tdTomato, was cut out of pCAGGS-I-oChIEF-ttdTomato-I-
WPRE (gift of R. Tsien) and ligated into pCAGGW-Chr2-Venus-
AAV backbone (donated by K. Svoboda; Addgene #20071) at
Age I and Xho I sites. This pAAV cis-plasmid was sent to Vector
Biolabs for large-scale production of the AAV (AAV-ChIEF-
tdTomato) (serotype 2/1, titer = 2.0 × 10^12 VG/ml).

For virus delivery, mice ranging in age between postnatal day
(P) 0–16 were deeply anesthetized with isoflurane. An incision
was made along the scalp, and a small hole created in the skull above
the visual cortex. Virus (1–2 μl total volume) was delivered via
a glass micropipette (placed in the deep layers of visual cortex)
using air pressure applied from a Picospritzer II (General Valve
Corporation, 5–10 psi, 2–5 msec duration, ±1 pulse per sec).
Typically two injections were made in each hemisphere and gave
rise to widespread expression throughout layer VI of the visual
cortex. Post-surgery, animals were carefully monitored for proper
wound healing, assessed for signs of pain or distress and
administered carprofen (5 mg/kg every 24 hours) and buprenor-
phine (0.1 mg/kg every 12 hours) for up to 3 days.

Slice Preparation and in vitro Recording

Ten to 14 days following injection of the AAV, mice were
deeply anesthetized with isoflurane and rapidly decapitated.
The brain was removed, hemisectioned, and immersed in an ice cold
oxygenated sucrose cutting solution containing (in mM): sucrose
230, KCl 2.5, CaCl_2 2, MgCl_2 6, NaHPO_4 1, NaHCO_3 25,
neurons were visualized on an upright microscope (Olympus BX51WI) equipped with both DIC optics and filter sets for visualizing GFP (Chroma 49002) and tdTomato (Chroma 49005) using a 10X or 60X water immersion objective (Olympus USA) and a CCD camera. Patch electrodes were pulled vertically in two stages from borosilicate glass and filled with (in mM): KMeSO4 135, NaCl 8, MgATP 2, NaGTP 0.3, HEPES 10, K2-BAPTA 0.1. The final tip resistance of filled electrodes was 3–6 MΩ. Whole-cell current and voltage clamp recordings were made using a Multiclamp 700B amplifier (Molecular Devices), filtered at 3–10 kHz, digitized at 10–100 kHz through an A/D board (National Instruments PCI-6221) and stored directly on a computer. Data acquisition and analysis was accomplished by using Strathclyde Electrophysiology Software (Whole Cell Analysis Program V3.8.2). Pipette capacitance, series resistance and whole cell capacitance were carefully monitored and compensated electronically during the recording. Voltage clamp recordings were performed without series resistance compensation. Recordings were made from cells with a resting membrane potential between −55 and −75 mV and a series resistance between 10 and 25 MΩ. Only experiments in which series resistance remained relatively stable (<25% change) were included for analysis. No significant differences in series resistance were found between different cell types (relay cells, 14.2 ± 5.1; TRN neurons, 14.0 ± 4.6; dLGN interneurons, 15.4 ± 2.6.1 MΩ; one-way ANOVA, F = 0.55, p > 0.5). Membrane potentials have been corrected for the calculated error resulting from the liquid junction potential (13 mV). Unless otherwise noted, the holding potential was −70 mV.

For photoactivation, light from a 150W Xenon arc lamp was bandpass filtered (450–490 nm; EGFP filterset, HQ470/40X exciter, Chroma 41018), and reflected into a 10X or 60X objective. This produced a spot of blue light onto the submerged slice with an approximate diameter of 2.2 or 0.45 mm, respectively. In some experiments, the epifluorescence apertures were adjusted to produce a small spot of light through the 60X objective with an approximate diameter of 25 μm. Light was gated using a shutter (Uniblitz VS25, Vincent Associates) in which pulse duration and frequency were under computer control. For repetitive stimulation, pulse duration was between 2–7 msec.

For recordings designed to assess the underlying pharmacology of synaptic events, a number of different ligand or voltage-gated ion channel antagonists were bath applied. The GABA_A and GABA_B antagonists, bicuculline methochloride (50 μM, Ascent Scientific Asc110) and CGP 55845 (1 μM, Tocris 1248) were used to block inhibitory postsynaptic activity. The AMPA and NMDA antagonists, NBQX (10 μM), Ascent Scientific Asc046 and APV (50 μM, Ascent Scientific Asc003) were used to block excitatory events. The metabotropic glutamate receptor 1z (mGluR1z) antagonist, LY367385 (100 μM, Tocris 1237) and the mGluR5 antagonist, MTEP (100 μM, Tocris 2921) were used to block Group I mGluRs. Nimodipine (10 μM, Tocris 0600) was utilized to block L-type Ca^2+ channels, while the voltage-gated Na^+ channel blocker tetrodotoxin citrate (TTX) (1 μM, Tocris 1069) was used to abolish action potential-mediated synaptic transmitter release.

The amplitude of synaptic responses was measured from baseline values obtained just prior to photostimulation. Evoked responses <6 pA were below our detection limit (set at ~2 times the average RMS noise) and thus excluded from the analysis. To quantify the degree of facilitation in responses evoked by repetitive trains of light, the amplitude of the n-th response was divided by the amplitude of the initial response, and then multiplied by 100. Typically, amplitude values were based on the average of 3–7 stimulus presentations. Descriptive and inferential statistics were performed using Prism 5 (Graphpad) and pCLAMP 9 (Molecular Devices).

**Histology**

In some recordings, Biocytin (0.1–0.2% w/v; Molecular Probes B1592) was included in the patch electrode and allowed to diffuse into the cell during recording. After the conclusion of recordings, the slice was placed in a fixative solution consisting of 4% paraformaldehyde in phosphate-buffered saline (PBS). Slices were kept in this solution overnight at 4 °C, and then washed in PBS. For labeling, slices were treated with 0.1% Triton X-100 and Alexa Fluor 647 conjugated strepavadin (1:1000, Invitrogen S21374) in PBS for 24 hours. Slices were then rinsed with PBS, mounted with Prolong Gold (Invitrogen P36930) and cover slipped. Epifluorescent images of fixed slices and filled cells were collected with a Nikon E600 upright microscope, and in some cases, confocal images were collected with a Zeiss LSM 510 Meta confocal microscope.

**Results**

**Expression of ChIEF-tdTomato in CT Neurons**

Targeted injections of the AAV construct containing the coding sequence for ChIEF-tdTomato into the deep layers of the visual cortex of golli-t-GFP mice resulted in strong expression in layer VI neurons (Fig. 2A). Indeed, there was a high degree of overlap between t-GFP labeled neurons (Fig. 2A, left), which are found throughout layer VI and project their axons into the thalamus [32], and regions that expressed ChIEF-tdTomato (Fig. 2A, right).

To confirm that we could reliably activate layer VI neurons with blue light, whole-cell recordings were made from visually identified pyramidal cells expressing ChIEF-tdTomato (n = 4). Representative recordings are shown in Fig. 2B-C. In these experiments, light-evoked activity was isolated by bath applying the glutamate receptor antagonists NBQX (10 μM) and APV (50 μM) as well as the GABA receptor antagonists bicuculline (50 μM) and CGP 55845 (1 μM). Long single pulses of blue light (500 ns) focused on the soma, resulted in a sustained depolarization (20–50 mV) and a train of action potentials that lasted throughout the period of light stimulation (Fig. 2B, upper trace). The depolarization exhibited relatively fast kinetics that corresponded to the onset and termination of the light pulse. Furthermore, corresponding voltage clamp recordings (Fig. 2B, lower trace) revealed that photocurrents were large (250–300 pA), had relatively fast kinetics and showed little inactivation. To ensure that action potentials could be initiated in axons, we presented small spots of blue light (~25 μm diameter) within the white matter, ~400–700 μm from the patched cell. As shown by the example in Fig. 2C, a train of pulses (10 Hz; 2 ms duration, 20 pulses) gave rise to repetitive spike firing. The expanded traces in Fig. 2C, reveal that each pulse evoked a single, fast rising action potential. Taken together these recordings demonstrate that light
activation of ChIEF expressing neurons results in strong levels of depolarization that can lead to reliable action potential firing.

dLGN Relay Cells

Cortical injections of AAV-ChIEF-tdTomato also gave rise to robust expression in CT axons innervating the dorsal thalamus. We routinely observed widespread expression of ChIEF-tdTomato that overlapped with t-GFP throughout the dLGN (Fig. 3A). Importantly, no somatic labeling was observed, indicating that expression in the thalamus was restricted to CT axons and their terminal fields. As shown in DIC and fluorescence images from an in vitro slice in Fig. 3B, a dense plexus of labeled axons and terminals could be seen surrounding somata that were targeted for recording.

Whole-cell recordings were obtained from a total of 76 relay neurons between P10–30. Under DIC optics, relay cells could be distinguished from interneurons by their large round somata (Fig. 3B). Their identity was further verified by their electrophysiological properties and in many cases (n = 50) by their dendritic morphology, which was reconstructed from biocytin fills conducted during the recording. As shown in Figs. 3C and 5B, relay cells were readily distinguished from interneurons, having type I or class A morphology that consisted of a thick unbranched axon, large round somata, and multipolar dendritic arbors with as many as six to seven primary dendrites [36–39].

At late postnatal ages (P21–30), when dLGN cells are considered adult-like [38,39] and well after the time CT axons innervate the dorsal thalamus [32], repetitive blue light stimulation evoked large and reliable excitatory postsynaptic potentials (EPSPs) and currents (EPSCs) that exhibited strong facilitation. Representative examples are shown in Fig. 3D–E, in which a 10 Hz (20 pulse) train of blue light generated a sequence of action potentials. Below the spike train are expanded traces of representative action potentials evoked by the 2nd (a), 9th (b) and 19th (c) pulse. Each pulse generated a single, fast rising action potential. For B and C, responses were recorded in the presence of NBQX (10 μM), APV (10 μM), bicuculline (50 μM) and CGP 55845 (1 μM).

To quantify the degree of facilitation evoked by repetitive CT stimulation, we used a 10 Hz, 20 pulse-train of blue light (Fig. 4A, top). Even responses to trains of this length continued to exhibit strong facilitation. Fig. 4B summarizes the responses of 31 relay cells by plotting the degree of facilitation as a function of stimulus number. Responses grew rapidly, but saturated by the 13th pulse (repeated measures ANOVA, F = 48.87; Dunnett’s post hoc test; pulses 1–12 vs 20, all p values <0.05; pulses 13–19 vs pulse 20, all p values >0.05), reaching a peak amplitude that was 1351±171.1% of the initial EPSC. To confirm these light-evoked EPSCs were due to the release of glutamate at CT terminals, we

Figure 2. ChIEF-tdTomato expression in the visual cortex of the golli-t-GFP mouse. A) Expression of τ-GFP (left) and ChIEF-tdTomato (right) in a 300 μm thick slice that was fixed overnight after in vitro recording. Note the correspondence of τ-GFP and tdTomato expression in layer VI. Because the tdTomato expression was so high in somata, labeled dendritic processes are obscured in the photomicrograph. Regions corresponding to different cortical layers are delineated in roman numerals. B) Current (above) and voltage clamp (below) responses of a layer VI neuron evoked by a single 500 ms pulse of blue light (blue bar) were large (−30 mV, −400 pA) and closely matched stimulus duration. In current clamp (V_m = −70 mV), a train of action potentials rises to the top of the sustained depolarization, while in voltage clamp (V_m = −70 mV) the light-evoked current persisted throughout the duration of the pulse showing little if any inactivation. Scale bar = 100 μm. C) Current clamp responses of a different neuron (V_m = −78 mV) to a small spot of blue light (25 μm diameter) that was directed at CT axons in the white matter about −500 μm from the patched cell. A 10 Hz (20 pulse) train of blue light generated a sequence of action potentials. Below the spike train are expanded traces of representative action potentials evoked by the 2nd (a), 9th (b) and 19th (c) pulse. Each pulse generated a single, fast rising action potential. For B and C, responses were recorded in the presence of NBQX (10 μM), APV (10 μM), bicuculline (50 μM) and CGP 55845 (1 μM).

doi:10.1371/journal.pone.0045717.g002
bath applied the AMPA receptor antagonist NBQX (10 μM), which in some cases was followed by the NMDA receptor blocker APV (50 μM). An example of light-evoked responses recorded before and after antagonist applications is shown in Fig. 4A. Overall, NBQX greatly reduced the response across each successive pulse (e.g., 91.8 ± 1.4% at the 18th pulse, n = 9), while the subsequent application of APV nearly abolished the remaining response (99.5 ± 0.2% of control, n = 6).

To study the development of CT responses in relay cells, cortical injections of AAV-ChIEF-tdTomato were made at early postnatal ages (P0, 4 and 12) and in vitro recordings were then conducted at P10 (n = 22), P14 (n = 16), and P22 (n = 7). Such timing corresponded to ages when CT projections begin to innervate the dorsal thalamus (P4–7) [32] and form synapses with dLGN relay cells (P7–14) [5]. As shown in Fig. 5A, by utilizing the golli-τ-GFP mouse we could verify that at the earliest age of recording (P10), CT arbors innervate nearly the entire dLGN and express high levels ChIEF-tdTomato. Representative examples of biocytin filled relay cells and corresponding CT-evoked responses are shown in Fig. 5B–C. Light-evoked responses were evident as early as P10 and the initial response to the stimulus train (10 Hz, 20 pulses) was similar in amplitude to those recorded at P14 and P22 (P10, 25.9 ± 5 pA; P14, 28.9 ± 10 pA; P22, 27.9 ± 9.3 pA; one-way ANOVA, F = 0.1, p > 0.9). However, maximal amplitude increased with age (P10, 24.8 ± 3.7 pA; P14, 75.5 ± 15 pA; P22, 288 ± 103.1 pA; one-way ANOVA, F = 15.77; tukey’s post hoc test;
P10 vs P14, p>0.4; P10 vs P22, p<0.0001; P14 vs P22, p<0.0001) and occurred at different stimulus intervals (Fig. 5D). For example, for both P10 and P14, the maximal amplitude occurred as early as the 2nd or 3rd pulse, whereas at P22, peak values were evident near the end of the stimulus train (18–20th pulse, see also Fig. 4B). Moreover, as shown in the summary plots of Fig. 5D, at P10 and P14 facilitation was transient and relatively weak. For example, at P10 facilitation peaked by the 3rd stimulus pulse (160.3±8.5%) and returned to near baseline values by the end of the stimulus train (117.1±7.6%, Fig. 5D, left). Similarly, at P14 facilitation was still weak, peaking around the 4th pulse (204.6±17.8%, Fig. 5D, middle), but sustaining these levels throughout the remainder of the

Figure 4. Facilitation and pharmacology of optically-evoked CT EPSCs in dLGN relay neurons. A) Example of EPSCs recorded from a relay neuron (V_h = −70 mV) evoked by a 10 Hz train of blue light pulses (20 pulses, blue bars). Bath application of NBQX (10 μM) greatly reduced the amplitude of evoked currents while subsequent application of APV (50 μM) abolished the remaining response. B) Summary plot showing the degree of facilitation of light-evoked CT EPSCs as a function of stimulus number for all relay neurons (n = 31). To calculate the percent facilitation, the amplitude of the nth response was divided by the amplitude of the initial response, and multiplied by 100. Each point represents the mean and bars indicate ±SEM.

doi:10.1371/journal.pone.0045717.g004
Figure 5. ChIEF-tdTomato expression and light-evoked responses in relay cells of the dLGN at different postnatal (P) ages. A) Comparison of t-GFP (left) and ChIEF-tdTomato (right) in a slice containing dLGN at P10. Arrows highlight a dorsolateral region just beneath the optic tract that lacks label, indicating that CT arbors have not yet innervated this area. Even at this age, cortical injections gave rise to a high level of ChIEF-tdTomato expression that matched the pattern of GFP-labeling. B) Z-stack projection images of relay cells at P10, P14 and P22 that were filled with...
biocytin during recording. Scale bar = 25 μm. C) CT-evoked responses from individual relay neurons to a 10 Hz train (20 pulses, blue bars) at the same ages shown in B. (D) Below each response is the corresponding summary plot showing the degree of facilitation (mean±SEM) as a function of stimulus number for the total number of neurons recorded at each age (P10, n = 22; P14, n = 16; P22 n = 7). For P10 and P14 error bars are too small to be seen. For P22, additional recordings were made from those summarized in Fig 3.

doi:10.1371/journal.pone.0045717.g005

train (20th pulse, 209.0±22.0%). As noted earlier, the degree of facilitation at late ages (>P21) was far greater (see Fig 4B). For comparison, at P22 facilitation grew rapidly and persisted throughout the train, reaching a value of 1192.0±324.1% (n = 7) of the initial amplitude (Fig 5D, right).

**TRN Neurons**

To examine CT responses in GABAergic TRN neurons, we made cortical injections of AAV-ChIEF-tdTomato in mature (P20–30) GAD65-GFP mice. Using a horizontal slice for recording, we readily observed strong expression ChIEF-tdTomato in CT axons and terminal fields that overlapped with GAD65-GFP neurons residing in the visual sector of the TRN (Fig 6A). Targeted recordings with biocytin filled electrodes revealed that these GFP positive cells (n = 15) had fusiform-shaped somata that were confined to the boundaries of TRN, and either bipolar or multipolar primary dendrites (Fig 6B).

Similar to the dLGN relay cells, TRN neurons also exhibited strong facilitating responses to repetitive stimulation (Fig 6C–D). Additionally, at hyperpolarized membrane potentials EPSPs were capable of eliciting LT Ca²⁺ spikes and burst responses (n = 8; Fig 6C). The summary plot in Fig 6E for TRN neurons shows that responses grew quickly, began to plateau by the 12th pulse (repeated measures ANOVA, F = 34.31; Dunnett’s post hoc test; pulses 1–11 vs 20, all p values <0.05; pulses 12–19 vs 20, all p values >0.05), and peaked at a value that was 721.8±88.9% (n = 24) of the initial EPSC. Bath application of NBQX (10 μM), and then APV (50 μM) revealed that responses were mediated almost entirely by AMPA and NMDA receptor activation (Fig 6D). NBQX reduced the response by 91.3±4.8% (n = 10) compared to control, while the addition of APV all but abolished the residual response (99.7±0.2% of control, n = 5).

**Comparison of dLGN Relay Cells and TRN Neurons**

While CT evoked EPSCs facilitate in both relay and TRN neurons, differences in the degree and kinetics have been reported [24,40]. To examine this in our sample of optically-evoked responses, we compared the initial and maximal amplitudes, maximal facilitation, and the rate of facilitation as a function of stimulus number (Fig 7A–B). We found the amplitude of the initial response was significantly greater for TRN compared to dLGN relay neurons (TRN, 94.2±25.6 pA; relay, 40.1±5.5 pA; two tailed t-test, p<0.05). However, maximal amplitudes (20th pulse) were not different from each other (TRN, 417.3±83.5 pA; relay, 465.5±72.4 pA; two tailed t-test, p>0.6). Thus, relay neurons exhibited about a 2-fold difference in the degree of facilitation compared to TRN neurons (relay, 1375.0±171.1%; TRN, 721.8±88.9%; Fishers exact test, p<0.01). To compare rates of facilitation, the degree of facilitation by stimulus number plots were best-fit by a single exponential function (Fig 7B). Indeed, response amplitudes grew faster for TRN cells (τa = 7.1) when compared to relay neurons (τa = 10.1).

**dLGN Intrinsic Interneurons**

To examine CT responses among interneurons within the dLGN, we made cortical injections of AAV-ChIEF-tdTomato in GAD67-GFP mice. In coronal slices, GFP positive interneurons were found throughout the dLGN and were surrounded by a dense plexus of tdTomato labeled CT arbors (Fig 8A). Recordings with biocytin filled electrodes showed that GFP positive neurons (n = 35) had morphology resembling intrinsic interneurons (Fig 8B), having type II or class B features that consisted of a bipolar appearance with long thin dendrites emanating from the opposite poles of small, fusiform somata [36,37,41]. These morphological features give rise to electrotonic isolation of dendrites, and as a result introduce potential space clamp problems during somatic recordings [21,42–44]. Since interneuron responses to CT stimulation obtained in the voltage clamp configuration were small and difficult to quantify (n = 5; Fig 8C), we chose to conduct recordings in current clamp mode, which had the advantage of being less sensitive to changes in access resistance and more likely permitting for the detection of small synaptic responses. Whole-cell current clamp recordings were obtained from 33 interneurons from mice that were between P20–30. Despite the widespread expression of ChIEF-tdTomato throughout the dLGN, light-evoked responses to stimulus trains comprised of 20 or 50 pulses presented at 10 Hz or 20 Hz were relatively weak and somewhat variable in size with peak amplitudes ranging from 2–15 mV. In fact, for many cells (n = 46) it was difficult to detect light-evoked events. It is unlikely that such failures were due to the degree of ChIEF-tdTomato expression in dLGN, since large responses could be routinely evoked in relay neurons recorded within the same slice (n = 8, data not shown). For those cells that responded to optical stimulation (n = 37), repetitive pulses produced a slow rising, graded depolarization. In many instances (n = 20), a series of small, unitary, fast EPSPs that corresponded to the temporal rate of stimulation could be discerned riding the crest of the prolonged depolarization (Fig 8D, inset).

To confirm these responses were synaptic in origin and dependent upon light-evoked action potentials generated in CT axons, we bath applied TTX (4 μM, n = 3). As illustrated in Fig 8D, TTX blocked both the fast EPSPs as well as the graded depolarization. Finally, to assess the underlying pharmacology of these events, we bath applied the glutamate antagonists NBQX (10 μM) and APV (50 μM) as shown in Fig 8E, and G, NBQX reduced the postsynaptic response to a value that was 37.2% of control. However, the subsequent application of APV had little if any effect on the remaining response (one-way ANOVA, F = 29.45, tukey’s post hoc test, control vs NBQX, p<0.05, n = 6; control vs NBQX/APV, p<0.05, n = 7; NBQX vs NBQX/APV, p>0.05, n = 3). To assess whether the remaining response could be due to activation of Group I metabotropic glutamate receptors (mGluRs) [25,45] we co-applied the mGluR1a antagonist, LY367385 (100 μM) along with the mGluR3 antagonist, MTEP (100 μM). However, as illustrated in Fig 8F–G, the NBQX/APV-resistant component of the depolarization persisted in the presence of these metabotropic antagonists (one-way ANOVA, F = 29.45, tukey’s post hoc test p>0.05, n = 3). During one recording we applied the L-type Ca²⁺ channel antagonist, Nimodipine (10 μM) which also failed to affect the remaining response, suggesting that the depolarization recorded at the soma was not mediated by Group I mGluRs nor voltage-gated L-type Ca²⁺ channels [46,47].
Figure 6. ChIEF-tdTomato expression of CT projections and light-evoked synaptic responses in GABAergic TRN neurons of the GAD65-GFP mouse. A) Superimposed fluorescence images of a 300 μm thick horizontal slice from a GAD65-GFP mouse that was fixed overnight following in vitro recordings. CT projections expressing ChIEF-tdTomato (red) can be seen in regions that contain GAD65-GFP positive TRN neurons (green). Areas in yellow depict overlap. P, posterior; M, medial. B) Confocal image stack of a TRN neuron filled with biocytin during recording. Scale bars = 25 μm. C) Example of a whole-cell current clamp recording (V_m = −85 mV) showing that repetitive stimulation led to EPSPs that could evoke LT Ca^{2+} spikes and burst responses (asterisks). Action potentials are truncated for clarity. D) Example of EPSPs (V_m = −70 mV) and corresponding EPSCs...
Discussion

Our results reveal that the chimera of channelrhodopsins 1 and 2, known as ChIEF, is a highly effective optogenetic tool that can be used to study synaptic responses in slice preparations where it is difficult to isolate or preserve long range neuronal connections. AAV delivery of ChIEF produced strong and widespread expression in layer VI neurons of the visual cortex, which included their descending axons and terminal arbors throughout the visual sector of the thalamus. Blue light stimulation of their somata or distal axon segments, produced large currents and spike firing that was well correlated with the duration and pattern of light delivery. In thalamic slices where CT axons have been severed from their cell bodies, activation of their terminal arbors in dLGN and TRN also gave rise to robust excitatory postsynaptic activity, indicating that ChIEF can be used as a viable alternative to electrical stimulation. In fact, the light-gated properties of ChIEF channels offer some key advantages over other more conventional forms of channelrhodopsins. Most notable are the fast open and close times, and lack of desensitization during

\[ V_{\text{th}} = -70 \text{ mV} \]

recorded from another GFP positive TRN neuron. Responses were evoked by a 10 Hz train of blue light (20 pulses, blue bars) and showed strong facilitation. Bath application of NBQX (10 µM) greatly reduced the amplitude of evoked currents while subsequent application of APV (50 µM) abolished the remaining response. E) Summary plot showing the degree of facilitation (mean±SEM) as a function of stimulus number for all TRN neurons (n = 31).

doi:10.1371/journal.pone.0045717.g006

Figure 7. Comparison of optically-evoked CT responses in relay cells and GABAergic TRN neurons. A) Summary plots showing the means (±SEM) for initial amplitude, maximal amplitude and maximum facilitation for relay cells and TRN neurons. Initial amplitude of relay cells was smaller, but had comparable maximal values thus exhibiting a greater degree of facilitation than TRN neurons (*p<0.05; **p<0.01). B) Plots for mean % facilitation (±SEM) as a function of stimulus number for relay and TRN neurons. Starting with the 2nd pulse, plots were best fit with a single exponential. A comparison of tau (relay, 10.1; TRN, 7.1) revealed that facilitation grew faster and plateaued for TRN neurons.

doi:10.1371/journal.pone.0045717.g007
Figure 8. ChIEF-tdTomato expression of CT projections in dLGN and light-evoked synaptic responses of interneurons in the GAD67-GFP mouse. A) Example of a coronal slice used for recording showing GAD67-GFP positive interneurons (left) and widespread expression ChIEF-tdTomato expression (right) throughout dLGN. B) Confocal image stack of an interneuron filled with biocytin during recording. Scale bars = 25 μm. C) Whole-cell current (top trace, V_m = −70 mV) and voltage (bottom trace, V_H = −70 mV) clamp recordings from an LGN interneuron showing that repetitive photostimulation of CT fibers (10 Hz, 50 pulses; blue bars), produced a slow rising depolarization and underlying inward current that peaked near the termination of stimulation. D) Graded depolarization (top trace) recorded from an interneuron (V_m = −70 mV) evoked by a 10 Hz train of blue light pulses (50 pulses, blue bars). Expanded trace (below) illustrates the small unitary EPSPs that ride the crest of the slower depolarization. Bath application of TTX (1 μM), abolished both the graded depolarization and unitary EPSPs (bottom trace). E) Example of another response evoked by repetitive stimulation (20 Hz, 50 pulses, top trace). Bath application of NBQX (10 μM) significantly reduced the amplitude of the graded depolarization (middle trace). The addition of APV (50 μM) had a negligible effect on the remaining depolarization (bottom trace). F) Excitatory response evoked by 20 Hz repetitive stimulation (50 pulses, top trace, V_m = −70 mV). Although bath application of NBQX (10 μM) and APV (50 μM) greatly reduced the response (middle trace), the addition of the Group I mGluR antagonists, MTEP and LY367381 had a no effect on the remaining depolarization (bottom trace). G) Summary plot of pharmacology experiments showing the mean reduction (±SEM) in the magnitude of the graded depolarization (pA x ms) expressed as a percentage of the control response. Application of NBQX reduced the response (n = 6) whereas the addition of APV (n = 7), or the addition of the Group I mGluR antagonists MTEP and LY367385 (n = 3) did not have any further effect on the remaining depolarization. (*p<0.05).

doi:10.1371/journal.pone.0045717.g008
prolonged photostimulation [33,48]. Such properties are especially useful for studies that involve repetitive stimulation, where a loss of current can lead to an artificial depression or blunting of responses [40,49].

The use of ChIEF also allowed us to explore the ontogeny of CT responses in thalamus. As a first step we focused on dLGN relay cells, since our recent EM analysis of synaptic profiles in the dLGN suggests that synaptic connections between CT terminals and relay cells first appear sometime between first and second postnatal week [5]. Indeed, large excitatory responses were observed as early as P10 but the degree of facilitation induced by repetitive stimulation was relatively weak and transient, and did not appear fully mature until sometime between P14 and P21. Taken together, these results suggest that the age related changes in facilitation likely reflect the maturation of processes that regulate the synaptic vesicle cycle [50,51]. One possibility is that synaptic proteins that aid in the recruitment of vesicles to active zones during high rates of stimulation have yet to mature. Most notable may be the synapsins, which in the dLGN have been found in corticothalamic terminals, and when genetically deleted lead to a decrease in short term facilitation of CT responses [26].

Our major findings with ChIEF showed that responses to CT stimulation varied among different classes of thalamic neurons. For relay cells of the dLGN and GABAergic cells of the TRN, repetitive stimulation produced a train of EPSCs that grew in amplitude with each successive pulse. These responses were mediated by ionotropic glutamatergic receptor activation and blocked by the bath application of NBQX and APV. While others have documented the involvement of metabotropic glutamate receptors [6,22,25,52], their activation was not evident under our recording conditions. Indeed, such activation in the dLGN requires high rates of stimulation (e.g., 50–100 Hz) [6,22,25]. Unfortunately, our attempt to use such frequencies during photostimulation did not yield consistent or sufficient levels of depolarization, thereby compromising our ability to conduct a thorough examination of the underlying pharmacology. Such failure seems related to the temporal fidelity of ChIEF, while superior to other opsins is still limited, especially when high rates of stimulation are employed [33,48,49].

While facilitation of CT responses has been well documented in relay cells and TRN neurons [22,24,26,27,52–57], we noted differences in the initial amplitude, rate of growth, and degree of facilitation between relay and TRN neurons. For relay cells, the initial response was smaller, grew slower but had maximal amplitude that was similar to TRN cells. As a result, the degree of facilitation was about 2-fold larger for relay cells than TRN neurons, a pattern that is consistent with the CT responses reported in ferret dLGN and TRN [24], and mouse VB and TRN [40]. These differences in the initial amplitude and degree of facilitation are in part due to their postsynaptic AMPA GluR subunit composition. CT-TRN synapses possess a higher density of GluR4 subunits [15] thereby providing for stronger AMPA mediated responses that are likely to desensitize more quickly during repetitive stimulation [24,58–61].

Thus, with a wider dynamic range, relay cells seem better suited to integrate the duration and intensity of CT input, a feature that has been implicated in synchronizing network activity between neocortex and thalamus during different behavioral states [60,62–64] or sensory processing [11,12]. Because of the inhibitory feedback connections between the TRN and the dLGN, CT activation of TRN neurons could further extend the dynamic range of relay cells, by dampening the initial excitation and spike generation of relay cells, thereby allowing them to show graded levels of depolarization during repeated stimulation. This balance in CT excitation and TRN feedback inhibition also seems critical for generation and maintenance of normal thalamocortical network oscillations. Indeed, weakening the excitatory strength of CT-TRN synapses results in increased CT relay cell excitability and the induction of abnormal paroxysmal activity [60].

In contrast to dLGN relay and TRN neurons, intrinsic interneurons in the dLGN responded to repetitive stimulation with a small, slow, graded depolarization, and in some instances, small unitary EPSPs rode the crest of this depolarization. Furthermore, a substantial component of synaptic responses recorded in interneurons remained after ionotropic glutamate receptor blockade. Pharmacological experiments suggested this residual response was not mediated by mGluRs [25] or the back propagation of voltage-gated L-type Ca2+ channels [46]. Although the underlying pharmacology of this response has yet to be determined, we cannot rule out the possibility that we failed to completely eliminate all postsynaptic glutamatergic transmission or that the remaining response reflected a photoelectrochemical artifact associated with blue light stimulation [65–67]. However, the latter seems unlikely since the residual depolarization persisted even when attempts were made to shield the recording pipette and ground wire.

The excitatory responses we recorded in interneurons after CT stimulation are similar to those reported in rat [25] but at odds with a recent report in the mouse dLGN, showing that paired or repetitive delivery of electrical shocks led to pronounced depression of excitatory responses [27]. Perhaps this discrepancy is related to the method of stimulation since electrical stimulation could potentially lead to inadvertent activation of interneuron circuits involving TRN and/or intranuclear axon collaterals of relay cells [13,68,69]. Nonetheless, the CT responses of interneurons evoked by photostimulation were much weaker than those recorded in dLGN relay and TRN neurons. In part, this could be due to the density and distribution of CT inputs onto these cell types. For example, interneurons receive far fewer CT inputs and these synapses appear to be located distally on the dendrites [4,19,20,70–71]. Because interneurons have very long, small-diameter dendrites, distal synaptic events are subject to electrotonic isolation [21,42–44]. Thus, the passive propagation of synaptic responses at these distant sites could lead to substantial attenuation and low-pass filtering of signals recorded at the soma. Nonetheless, it is clear that CT feedback leads to the excitation of intrinsic interneurons. Because of the inhibitory connections between interneurons and relay cells, such excitation would, as it does with the TRN, counteract the rapid initial CT induced depolarization of relay cells. Since feed-forward inhibition from interneurons onto relay cells contributes to their receptive field tuning [72–74], it is also conceivable that the improved stimulus specific tuning associated with CT activation could arise from the concerted CT activation of relay cells and interneurons [2,11,12,75]. Finally it is worth noting that interneurons can inhibit relay cells through specialized F2 terminals, which form dendrodendritic synapses [5,13,21,70,76]. Since these F2 terminals are prevalent on distal dendritic sites, such an arrangement allows for highly localized interactions that can occur independent of somatic interneuron activity [43,44,46,77].

In conclusion, these results reveal that optogenetics can be used effectively to examine long-range synaptic interactions in developing and mature circuits. When applied to the CT pathway they reveal that relay cells of dLGN neurons have a different excitatory profile than GABAergic TRN neurons and intrinsic interneurons. The concerted activation of GABAergic neurons during CT activation can further shape the excitatory responses of relay cells, extending their dynamic range during repetitive and
sustained periods of stimulation. Understanding these differences in the pattern of excitation for different thalamic cell types should provide additional insight about how CT feedback alters the activity of visual thalamic circuitry during different behavioral or pathophysiological states.

Acknowledgments

We would like to thank J.Y. Lin and R. Tsien for the generous gift of ChIEF, K. Svoboda for the gift of pACAGW-Chr2-Venus-AAV, A. T. Campagnoni for providing the golgi-t-GFP mice, R.N. El-Danaf for assistance with the biocytin labeling and confocal imaging and T.E. Krake for many helpful discussions and critical reading of the manuscript.

Author Contributions

Conceived and designed the experiments: CWDJ ARM WG. Performed the experiments: CWDJ WG. Analyzed the data: CWDJ WG. Contributed reagents/materials/analysis tools: KAB ARM. Wrote the paper: CWDJ ARM WG. Designed and created the AAV-ChIEF-edTomato plasmid: KAB.

References

1. Sherman SM, Guillery RW (2002) The role of the thalamus in the flow of information to the cortex. Philos Trans R Soc Lond B Biol Sci 357: 1655–1708.
2. Briggs F, Usrey WM (2008) Emerging views of corticothalamic function. Curr Opin Neurobiol 18: 403–407.
3. Eriji A, Van Horn SC, Bickford ME, Sherman SM (1997) Immunocytochemistry and distribution of parafunctional terminals in the lateral geniculate nucleus of the cat: a comparison with corticogeniculate terminals. J Comp Neurosci 377: 535–549.
4. Li J, Wang S, Bickford ME (2003) Comparison of the ultrastructure of cortico and retinal terminals in the rat dorsal lateral geniculate and lateral posterior nucleus. J Comp Neurol 460: 394–409.
5. Bickford ME, Sharsack A, D’Agger EK, Krahe TE, Kucek C, et al. (2010) Synaptic development of the mouse dorsal lateral geniculate nucleus. J Comp Neurol 518: 622–635.
6. McCormick DA, von Krosigk M (1992) Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors. Proc Natl Acad Sci U S A 89: 2774–2783.
7. Godsch DW, Vaughan JW, Sherman SM (1996) Metabotropic glutamate receptors switch visual response mode of lateral geniculate nucleus cells from burst to tonic. J Neurophysiol 76: 1800–1816.
8. Tsutsumo T, Kuretfeld OD, Legenda CR (1976) Functional organization of the corticofugal system from visual cortex to lateral geniculate nucleus in the cat (with an appendix on geniculo-cortical mono-synaptic connections). Exp Brain Res 32: 345–364.
9. Murphy PC, Silfio AM (1987) Corticothalamic feedback influences the generation of length tuning in the visual pathway. Nature 329: 727–729.
10. Przybyzteski AW, Gaska JP, Foote W, Pollen DA (2000) Striate cortex increases contrast gain of macaque LGN neurons. Vis Neurosci 17: 485–494.
11. Silfio AM, Jones HE, Gerstein GL, West DC (1994) Feature-linked synchronization of thalamic relay cell firing induced by feedback from the visual cortex. Nature 369: 479–482.
12. Andolino IM, Jones HE, Di Cristo G, Higashiyama H, Kuhlman SJ, et al. (2004) Experience and activity-dependent maturation of persomatoic GABAergic innervation in primary visual cortex during a postnatal critical period. J Neurosci 24: 9590–9611.
13. Lopez-Bendito G, Stueb K, Mohn Z, Mohr A, et al. (2004) Preferential origin and layer destination of GAD63/GFP corticointerneurons. Cereb Cortex 14: 112–123.
14. Rafols JA, Valverde F (1973) The structure of the dorsal lateral geniculate nucleus in the mouse. A Golgi and electron microscopic study. J Comp Neurol 150: 303–332.
15. Parnavelas JG, Mouyst EJ, Bradford R, Lieberman AR (1977) The postnatal development of neurons in the dorsal lateral geniculate nucleus of the rat: a Golgi study. J Comp Neurol 171: 401–499.
16. Vlaskamp A, Post J, van Wagenen A, Buwalda B, van den Noort A (1995) Synaptic mechanisms involved in the production of thalamic relay neuron activity. Vis Neurosci 8: 417–423.
17. Wang S, Bickford ME, Van Horn SC, Erisir A, Godsch DW, et al. (2001) Synaptic contacts of thalamic reticular nucleus terminals in the visual thalamus of the cat. J Comp Neurol 440: 321–341.
18. Weber AJ, Kalil RE, Behan M (1989) synaptic connections between corticogeniculate axons and interneurons in the dorsal lateral geniculate nucleus of the cat. J Comp Neurol 289: 156–164.
19. Montero VM (1981) Quantitative study of the functional connections of relay cells of the lateral geniculate nucleus. Exp Brain Res 39: 257–270.
20. Sherman SM (2004) Interneurons and triadic circuitry of the thalamus. Trends Neurosci 27: 670–675.
21. Turner JP, Salt TE (1998) Characterization of sensory and corticothalamic excitatory inputs to rat thalamocortical neurones in vitro. J Physiol 510: 829–843.
22. Granseth B (2004) Dynamic properties of corticogeniculate excitatory transmission in the rat dorsal lateral geniculate nucleus in vitro. J Physiol 556: 135–146.
23. Alexander GM, Fisher TL, Godsch DW (2006) Differential response dynamics of corticothalamic glutamatergic synapses in the lateral geniculate nucleus and thalamic reticular nucleus. Neuroscience 137: 367–372.
24. Gouras I, Cox CL (2006) Metabotropic glutamate receptors differentially regulate GABAergic inhibition in thalamus. J Neurosci 26: 13453–13453.
25. Kieland A, Erisir A, Walaas SI, Heggelund P (2006) Synapsis utilization differs among functional classes of synapses on thalamocortical cells. J Neurosci 26: 5786–5793.
26. Augustine S, Yanagawa Y, Heggelund P (2011) Cortical feedback regulation of input to visual cortex: role of intraganglionic interneurons. J Physiol 589: 2963–2977.
27. Sherman SM, Guillery RW (1996) Functional organization of thalamocortical relays. J Neurophysiol 76: 1367–1395.
28. Bickford ME, Wei H, Eisenbach MA, Chomsung RD, Sharsack AS, et al. (2008) Synaptic organization of thalamocortical axon collaterals in the prereticular geniculate and dorsal lateral geniculate nucleus. J Comp Neurol 508: 264–285.
29. Ulrich DJ, Cucchiario JR, Sherman SM (1980) The projection of individual axons from the parabasal region of the brain stem to the dorsal lateral geniculate nucleus in the cat. J Neurosci 8: 4565–4573.
30. Parent M, Descaries L (2006) Acetylcholine innervation of the adult rat thalamus: distribution and ultrastructural features in dorsolateral geniculate, parafascicular, and reticular thalamic nucleus. J Comp Neurol 511: 678–691.
31. Jacobs EC, Campagnoni G, Kampf K, Reyes SD, Calra V, et al. (2007) Visualization of corticofugal projections during early cortical development in a taa-GFP-transgenic mouse. Eur J Neurosci 25: 17–30.
32. Lin JY, Lin MZ, Steinbach P, Tsien RY (2009) Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys J 96: 1803–1814.
33. Chattopadhyaya B, Di Cristo G, Higashiyama H, Kuhlman SJ, et al. (2004) Experience and activity-dependent maturation of persomatoic GABAergic innervation in primary visual cortex during a postnatal critical period. J Neurosci 24: 9590–9611.
34. Rafols JA, Valverde F (1973) The structure of the dorsal lateral geniculate nucleus in the mouse. A Golgi and electron microscopic study. J Comp Neurol 150: 303–332.
35. Parnavelas JG, Mouyst EJ, Bradford R, Lieberman AR (1977) The postnatal development of neurons in the dorsal lateral geniculate nucleus of the rat: a Golgi study. J Comp Neurol 171: 401–499.
36. Jaffard-Maza R, Green E, Lo F-S, Bui K, Mills J, et al. (2005) Structural and functional composition of the developing retinogeniculate pathway in the mouse. Vis Neurosci 22: 661–676.
37. Krahe TE, El-Danaf RN, D’Agger EK, Heidenreich SU, Guido W (2011) Morphologically distinct classes of relay cells exhibit regional preferences in the dorsal lateral geniculate nucleus of the mouse. J Neurosci 31: 17437–17448.
38. Crompton DJ, Uchida H, Nurmok T, Anwari B, Tsiouris B, et al. (2006) Optogenetic control of thalamocortical relays. J Neurosci 32: 345–364.
39. Weber AJ, Kalil RE, Behan M (1989) synaptic connections between corticogeniculate axons and interneurons in the dorsal lateral geniculate nucleus of the cat. J Comp Neurol 289: 156–164.
40. Montero VM (1991) A quantitative study of synaptic contacts on interneurons and relay cells of the cat lateral geniculate nucleus. Exp Brain Res 86: 257–270.
41. Sherman SM (2004) Interneurons and triadic circuitry of the thalamus. Trends Neurosci 27: 670–675.
42. Turner JP, Salt TE (1998) Characterization of sensory and corticothalamic excitatory inputs to rat thalamocortical neurones in vitro. J Physiol 510: 829–843.
43. Granseth B (2004) Dynamic properties of corticogeniculate excitatory transmission in the rat dorsal lateral geniculate nucleus in vitro. J Physiol 556: 135–146.
Granseth B, Ahlstrand E, Lindström S (2002) Paired pulse facilitation of synaptic vesicle cycling as a distinctive feature of central glutamatergic synapses. Neuroscience 117: 7–18.

Sudhof TC (2004) The synaptic vesicle cycle. Annu Rev Neurosci 27: 509–547.

Casale AE, McCormick DA (2011) Dynamic properties of corticothalamic excitatory postsynaptic potentials and thalamic reticular inhibitory postsynaptic potentials in thalamocortical neurons of the guinea-pig dorsal lateral geniculate nucleus. Neuroscience 91: 7–20.

Lindström S, Wrobel A (1990) Frequency dependent corticofugal excitation of principal cells in the cat’s dorsal lateral geniculate nucleus. Exp Brain Res 79: 313–318.

von Krosigk M, Monckton JE, Reiner PB, McCormick DA (1999) Unique presynaptic and postsynaptic roles of Group H metabotropic glutamate receptors in the modulation of thalamic network activity. Neuroscience 141: 501–513.

Casale AE, McCormick DA (2011) Active action potential propagation but not synaptic transmission in visual thalamus. J Neurosci 31: 3322–3331.

Crandall SR, Cox CI (2012) Local dendrodendritic inhibition regulates fast synaptic transmission in visual thalamus. J Neurosci 32: 2513–2522.

Halassa MM, Sisjem JH, Ritt JT, Ting JT, Feng G, et al. (2011) Selective optical drive of thalamic reticular nucleus generates thalamic bursts and cortical spindles. Nat Neurosci 14: 1118–1120.

Ayling OGS, Harrison TC, Boyd JD, Goroshkov A, Murphy TH (2009) Automated light-based mapping of motor cortex by photosactivation of channelrhodopsin-2 transgenic mice. Nat Methods 6: 219–224.

Han X, Qian X, Bernstein JG, Zhou H-H, Franzesi GT, et al. (2009) Millisecond-timescale optical control of neural dynamics in the nonhuman primate brain. Neuron 62: 191–198.

Cardin JA, Castele M, Meleis K, Knoblich U, Zhang F, et al. (2010) Targeted optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression of Channelrhodopsin-2. Nat Protoc 5: 247–254.

Zhu JJ, Lo FS (1999) Three GABA receptor-mediated postsynaptic potentials in interneurons in the rat lateral geniculate nucleus. J Neurosci 19: 5721–5730.

Cox CI, Reichova I, Sherman SM (2003) Functional synaptic contacts by intracellular axon collaterals of thalamic relay neurons. J Neurosci 23: 7642–7646.

Hamos JE, Van Horn SC, Raczkowski D, Uhlich DJ, Sherman SM (1985) Synaptic connectivity of a local circuit neuron in lateral geniculate nucleus of the cat. Nature 317: 618–621.

Vidnyánszky Z, Hámiti J (1994) Quantitative electron microscopic analysis of synaptic input from cortical areas 17 and 18 to the dorsal lateral geniculate nucleus in cats. J Comp Neurol 349: 259–268.

Sillito AM, Kemp JA (1983) The influence of GABAergic inhibitory processes on the receptive field structure of X and Y cells in cat dorsal lateral geniculate nucleus (dLGN). Brain Res 277: 63–77.

Norton TT, Godwin DW (1992) Inhibitory GABAergic control of visual signals at the lateral geniculate nucleus. Prog Brain Res 90: 193–217.

Russeas O, Fjeld IT, Heggheld P (2000) Spatial summation and center–surround antagonism in the receptive field of single units in the dorsal lateral geniculate nucleus of cat: comparison with retinal input. Vis Neurosci 17: 855–864.

Cudeiro J, Sillito AM (1996) Spatial frequency tuning of orientation-discontinuity-sensitive corticofugal feedback to the cat lateral geniculate nucleus. J Physiol 490: 481–492.

Montero VM, Singer W (1985) Ultrastructural identification of somata and neural processes immunoreactive to antibodies against glutamic acid decarboxylase (GAD) in the dorsal lateral geniculate nucleus of the cat. Exp Brain Res 59: 151–165.

Crandall SR, Cox CI (2012) Local dendrodendritic inhibition regulates fast synaptic transmission in visual thalamus. J Neurosci 32: 2513–2522.