The primary visual cortex exhibits a late, long response with a latency of >300 ms and an immediate early response that occurs ~100 ms after a visual stimulus. The late response is thought to contribute to cortical functions such as sensory perception, iconic memory, working memory, and forming connections between temporally separated stimuli. However, how the visual late response is generated and organized is not completely understood. In the mouse primary visual cortex in vivo, we isolated long-delayed responses by using a brief light-flash stimulus for which the stimulus late response occurred long after the stimulus offset and was not contaminated by the instantaneous response evoked by the stimulus. Using whole-cell patch-clamp recordings, we demonstrated that the late rebound response was shaped by a net-balanced increase in excitatory and inhibitory synaptic conductances, whereas transient imbalances were caused by intermittent inhibitory barrage. In contrast to the common assumption that the neocortical late response reflects a feedback signal from the downstream higher-order cortical areas, our pharmacological and optogenetic analyses demonstrated that the late responses likely have a thalamic origin. Therefore, the late component of a sensory-evoked cortical response should be interpreted with caution.

Key words: bottom-up; patch-clamp; top-down; visual cortex
activity in mice (Zhang et al., 2014). However, this feedback modulation only accounts for a part of the response and much of the late component exists regardless of the top-down influence, which suggests that the late response is composed of inputs from pathways other than top-down feedback.

In this study, we examined the late response of V1 neurons to a brief short visual stimulus (a light flash). We demonstrated previously that V1 neurons exhibit a long latency response, as well as an early response, to a light flash and the late response modulates visual perception (Funayama et al., 2015). Here, we focused on the mechanisms underlying these V1 late responses. Specifically, we investigated how these V1 late responses are generated and organized in the visual nervous system. We initially investigated the neuronal circuit mechanism underlying the late response. We patch-clamped V1 layer 2/3 neurons in vivo and recorded their synaptic inputs in response to a brief light flash stimulus. We demonstrated that both excitatory and inhibitory inputs contribute to the late spike responses and that inhibition exerted more control over spike timing. Using pharmacological and optogenetic manipulations of neuronal activity in vivo, we demonstrated that the cortical late response occurs via thalamocortical inputs and emerges in the dLGN. Notably, no similar late response was identified in the retina. Therefore, the V1 late response is generated via a mechanism distinct from the early response.

Materials and Methods

Ethical approval. Animal experiments were performed with the approval of the Animal Experiment Ethics Committee at the University of Tokyo (approval number: 26–5) and according to the University of Tokyo’s guidelines for the care and use of laboratory animals. For human studies, the experimental protocol was approved by the Human Research Ethics Committee of the University of Tokyo (approval number: 24–3) and the Center for Information and Neural Networks (approval number: 1312260010). All participants provided oral and written informed consent and signed consent forms before each experiment.

Animal preparation for recordings. Postnatal day 35 (P35) to P42 male C57BL/6 mice (Japan SLC) were used in the animal experiments, as described previously in detail (Minamisawa et al., 2011; Ishikawa et al., 2014). The animals were housed in cages under standard laboratory conditions (a 12 h light/dark cycle and with ad libitum access to food and water). All efforts were made to minimize animal suffering and the number of animals used. For Figures 1, C and D, 5–A, C, 7, 8, and 9, recordings were obtained from urethane-anesthetized mice, whereas for Figures 1, A and B, 3, 4, 5D, and 6, recordings were obtained from awake mice. For urethane-anesthetized recordings, the animals were anesthetized with urethane (1.0–1.5 g/kg, i.p.). Anesthesia was confirmed by the lack of the paw-withdrawal, whisker-movement, and eye-blink reflexes. The head skin was then removed and the animal was implanted with a metal head-holding plate. A craniotomy (1 × 1 mm²) was performed over V1 (centered at 3.5 mm posterior to bregma and 2.0 mm ventrolateral to the sagittal suture) or over the dLGN (centered at 1.70 mm posterior to bregma and 3.5 mm ventrolateral to the sagittal suture) and the dura was removed surgically. For awake recordings, the surgery and head-hold training were conducted with the same protocol as used in our previous study (Funayama et al., 2015). Briefly, animals were anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) before the head-holding plate surgery and before the craniotomy. Then, after a few days of recovery, animals were trained every day until they could remain immobile under head fixation for >2 h. For the dLGN recording, regions of the primary somatosensory cortex and the hippocampus were carefully removed by aspiration and the top surface of the dLGN was exposed. The exposed surface was covered with 1.7–2.0% agar at a thickness of 0.5 mm. Throughout the experiment, a heating pad was used to maintain a rectal temperature of 37°C and 0.2% lidocaine was applied to the surgical region for analgesia.

Visual stimulation. Visual stimuli were generated in custom-written MATLAB routines (The MathWorks) with Psychtoolbox (Brainard, 1997) extensions. For the mouse experiments, a 17-inch TN-LCD monitor (refresh rate = 60 Hz) was placed 30 cm away from the right cornea so that it covered 38.8° horizontally and 29.6° vertically of the mouse visual field. For the flash stimulus, a white screen (contrast: 100%) was presented and its duration was set to 50 ms. The flash stimuli were presented 20–50 times at intervals of 8–10 s and a gray screen was presented during the interval period. For the human experiments, a visual stimulus was projected from a gamma-corrected LCD projector (Ban and Yamamoto, 2013) to the translucent screen and the participants viewed the screen through a front-surfaced mirror. The viewing distance was 46 cm and the visual angle of the screen was 51.9 × 30.6°. The background color of the display was set to black (0.37 cd/m²). A fixation cross was presented continuously in the center of the screen and the participants were asked to fix their eye position on the cross throughout the task. The start of a trial was indicated by a color change of the fixation cross from red to white (20.0 cd/m²). After 2000 ms, the whole screen was changed to white (169 cd/m²) for 50 ms and subsequently returned to the black screen with the fixation cross. At 4000 ms after the flash, the color of the fixation cross changed to green, indicating the end of the trial. The participants were asked not to blink during the trial. The participants initiated the trial at their own pace by pressing a key with their right index finger, with a minimum intertrial interval of 5 s. One scan block consisted of 30 trials and the participants performed five (six participants) or six (two participants) blocks during the measurement.

Electrophysiology. The signal was amplified using a MultiClamp 700B, analyzed using pCLAMP10.1 (Molecular Devices), and digitized at 20 kHz. The data were reduced to 2 kHz and analyzed offline using custom-written MATLAB routines. Patch-clamp recordings and local field potential (LFP) recordings were obtained at depths of 150–350 μm from the V1 surface, which corresponds to neocortical layer 2/3, or at 50–200 μm from the dLGN surface using borosilicate glass electrodes (3.5–6.5 MΩ) that were pulled with a P-97 puller (Sutter Instruments). The electrode tips were lowered perpendicularly into V1 with a DMX-11 electric manipulator (Narishige). For the cell-attached recordings, the pipettes were filled with artificial CSF (aCSF). For the whole-cell current-clamp recordings, the intrapipette solution consisted of the following (in mM): 130 K-glucuronate, 10 KCl, 10 HEPEs, 10 Na2-phosphocreatine, 4 Mg-ATP, 0.3 Na2GTP, 0.05 Alexa Fluor 594 hydrazide, and 0.2% biocytin, adjusted to pH 7.3. For the voltage-clamp recordings, the intrapipette solution consisted of the following (in mM): 130 CsMesO4, 4 tetraethylammonium-Cl, 10 HEPEs, 10 Na2-phosphocreatine, 0.5 EGTA, 4 MgATP, 0.3 Na2GTP, 2 QX-314, and 0.05 Alexa Fluor 594. For 20 kHz using a PCI-6024E data acquisition board (National Instruments) in a real-time Linux environment. During conductance injection, the gain was controlled to induce spikes from patch-clamped neurons.
Wavelet transform of LFP responses. We measured the time-varying oscillatory power of the LFP responses using a complex Morlet wavelet (center frequency of 1.5 Hz, bandwidth parameter of 1) defined as follows:

\[
\tilde{x}(t) = \int_{-\infty}^{\infty} x(t) \varphi_{a,b}(t) dt \quad \text{(Continuous wavelet transform)}
\]

The mother wavelet is defined as follows:

\[
\varphi(x) = \sqrt{\pi f_c} e^{\frac{2\pi itx}{a^2}} e^{-\frac{\pi^2 x^2}{a^2}} \quad \text{(Complex Morlet wavelet)}
\]

Where \( a \) is the scale factor, \( b \) is the shift (1/sample rate), \( f_c \) is the center frequency, and \( \varphi \) is the bandwidth parameter. The scale factor \( a \) was set to frequencies between 1 and 60 Hz with intervals of 1 Hz.

**Results**

**Flash-induced late response in mouse V1 layer 2/3**

In our previous study (Funayama et al., 2015), we visually stimulated awake mice with a brief presentation of a sinusoidal grating as a flash stimulus. Here, we used a simpler stimulus, a white-screen plain flash, to unmask the basic principles for the functional organization of flash-evoked cortical late responses. To confirm that a white-screen flash induces a similar late response in V1, we first conducted whole-cell current-clamp recordings from V1 layer 2/3 using a potassium-based intracellular solution (Fig. 1A, B). Consistent with our previous findings, the white-screen flash induced biphasic voltage responses \( V_m \) that were composed of a fast, transient depolarization (early response) and then late, prolonged depolarization (late response). The biphasic responses were identified in all 30 neurons tested in 26 mice. On average, the early depolarization had a peak amplitude of 1.3 ± 0.5 mV (mean ± SEM of 30 neurons) and a peak latency of 70.0 ± 7.3 ms, whereas the slow depolarization persisted from ~0.4 to 2.0 s after the stimulation and had a peak amplitude of 9.3 ± 1.1 mV. The variety in the peak timing of the late responses among cells resulted in seemingly lower late \( V_m \) peak amplitudes in the average trace shown in Figure 1B. These depolarizations were occasionally accompanied by action potentials. Specifically, 2 of 30 neurons fired spikes during the early depolarization period (0–0.2 s after the flash), and the mean firing rate of these two early spiking cells was 3.8 Hz. During the late depolarization period (0.4–2.0 s), 15 of 30 neurons exhibited firing and the mean firing rate of the late spiking cells was 0.59 ± 0.15 Hz. Note that the spontaneous firing rate during the preflash baseline period was 0.08 ± 0.12 Hz.

To determine whether flash-induced biphasic responses depend on the brain state of the mouse, we conducted whole-cell current-clamp recording and LFP recording (Fig. 1C–E) simultaneously under anesthesia. We observed similar biphasic \( V_m \) responses even in anesthetized mice. Consistent with previous
Figure 1. A flash of light induces a delayed response in layer 2/3 neurons of the mouse primary visual cortex. Electrophysiological recordings were made in both awake (A, B) and urethane-anesthetized (C–E) mice. A, Whole-cell current-clamp recordings were acquired from V1 layer 2/3 neurons in awake, head-restricted mice in which the contralateral eyes were presented with full-field white flashes at pseudorandom intervals of 8–10 s for 50 trials. Top, Five representative trials of \( V_m \) responses in a pyramidal neuron. Bottom, Mean subthreshold \( V_m \) responses across all 50 trials in the same neuron. The gray area represents the SD. B, Mean ± SD subthreshold \( V_m \) responses of all 30 neurons recorded from 26 mice. C, Raw recording traces of LFPs (top) and \( V_m \) responses of a single pyramidal cell (middle) were simultaneously acquired from layer 2/3 of mouse V1. Of 50 trials of flash stimuli presented to the contralateral eye, five randomly selected trials are shown. Bottom, Mean ± SD subthreshold \( V_m \) responses of the example neuron. D, Top, Mean powers of LFPs were computed using the wavelet transform. Bottom, Mean ± SEM of the LFP powers for all 28 LFP recordings from 28 mice. E, Histogram of spike responses for all eight multiunit recordings from eight mice.

Figure 2. A flash of light induces a late response in the human occipital cortex. A, Left, Topographical plot of the MEG response in the occipital channels. Representative data from a single participant are shown. Color indicates the intensity of the signal at 40–65 ms after the flash. Right, Time course of the MEG signal recorded in the occipital channel shown by the arrow. Data represent the means of 120 trials. B, Average ± SD time course data of the most active occipital channel across all eight participants. Periods indicated in red represent the time point at which the signal increased significantly compared with the period before the flash \((p < 0.05, \text{FDR corrected})\). C, dSPM analysis using the average MEG signal recorded from eight participants.
studies, the $V_m$ fluctuations of nearby neurons are reflected in LFPs (Deweese and Zador, 2004; Haider et al., 2006; Poulet and Petersen, 2008). A flash induced a transient early increase and a persistent late increase in the LFP powers at frequencies of 1–60 Hz (Fig. 1D). Furthermore, multiple units in the LFPs also exhibited biphasic increments in the firing rates (Fig. 1E). Therefore, a simple white-screen flash induced biphasic responses in a large population of V1 neurons.

We next focused on the trial-to-trial variability of the late responses. Because the LFP powers between 1 and 60 Hz fluctuated in a similar way (Fig. 1D, bottom), we extracted the mean gamma powers (30–60 Hz) of individual stimulation trials from 28 LFP recording in 28 mice. For each trial, we analyzed the gamma-frequency power during the late responses and compared it with the preflash gamma power. We observed that the late gamma power in a given trial was negatively correlated with the preflash baseline ($R^2 = -0.19 \pm 0.23$, mean $\pm$ SD of 29 mice; $p = 1.7 \times 10^{-4}$, $t_{28} = 4.34$). Therefore, the late response was modulated by the brain state before the flash stimulus.

**Flash-induced late response in the human occipital cortex**

We also used MEG to investigate whether a plain flash induces early and late complex responses in humans. In each participant, the location of the MEG sensor that exhibited the highest activity during the period of 40–65 ms after a light flash was defined as the occipital region of the brain (Fig. 2A). These sensors most likely reflected the initial evoked field that originated in the early visual cortex. In 8 participants, we tracked the evolution of activity in the sensors over time and identified a significant reactivation of the sensor during the period 0.4–1 s after the flash (Fig. 2B). We further evaluated the biphasic responses in the occipital region using dSPM analysis, which estimates the source localization of the response signal (Fig. 2C). This approach revealed that early and late responses were evident in the occipital region. Therefore, the human activity pattern resembles the biphasic responses of the mouse V1. Interestingly, during the onset time of the late responses (such as a time frame of 450 ms), brain regions other than the occipital cortex appeared silent and the late responses seemed to be initiated from the visual cortex, at least at the cortical level.

**V1 late synaptic inputs**

We subsequently returned to the V1 cortex of awake mice and sought to determine how the V1 late response is generated. We first examined the synaptic inputs underlying the depolarization of the V1 late response. We obtained whole-cell voltage-clamp recordings using a cesium-based internal solution. Individual trial traces and the averaged traces of the EPSC-dominant and IPSC-dominant currents are shown in Figure 3, A and B, respectively. In both traces, the early and late responses were evident.
Therefore, the biphasic responses were associated with a coordinated increase in excitation and inhibition. The averaged EPSC traces exhibited a seemingly outward transient current after the early flash response. This outward current may simply be caused by a reduction in the tonic excitation that was present during prestimulus basal conditions; however, voltage-clamp recordings, especially when conducted in vivo, are vulnerable to the space-clamp problem, which might also underlie the outward current.

We initially analyzed the background synaptic conductances before the presentation of the flash stimulus. Individual traces indicated that during the preflash baseline conditions, the IPSCs often occurred as a form of large, barrage-like synaptic inputs compared with the EPSCs (Fig. 3A). To quantify this difference in the input patterns, we calculated the SD of the membrane potential fluctuations during the preflash period. Consistent with the eye inspection, the \( G_e \) had a significantly larger SD than the \( G_i \) (Fig. 3C; \( * * p = 9.79 \times 10^{-11}, t_{(149)} = 7.80, G_e \) vs \( G_i \), Student’s t test, \( n = 150 \) trials from 7 cells from 7 mice). The amplitudes of the individual IPSC barrages ranged from \( \sim 100 \) to 300 pA and were larger than the amplitudes of the unitary IPSCs evoked by single GABAergic synapses (Yoshimura and Callaway, 2005; Ren et al., 2007), which suggests that they were produced by synchronized inhibitory inputs from presynaptic interneuron ensembles.

We subsequently analyzed the changes in the \( G_e \) and \( G_i \) (\( \Delta G_e \) and \( \Delta G_i \), respectively) during the late responses that were identified 0.4 – 2.0 s after the flash. The membrane potential may be imperfectly clamped; therefore, we calculated the change in conductance, rather than the absolute conductance values, by subtracting the prestimulus mean value. This calculation was also expected to cancel out the effect of the cesium-based solution on the membrane potential given that cesium ions block ion channels associated with the resting conditions. The time-evolution plot of the mean \( \Delta G_e \) and \( \Delta G_i \) across all 7 cells indicated that the \( \Delta G_e \) was approximately proportional to the \( \Delta G_i \) for the entire period of 1.6 s (Fig. 3D), indicating that the excitatory and inhibitory inputs were co-tuned at a given time point. However, for the individual trials, the pattern of flash-induced conductance changes differed between the \( G_e \) and \( G_i \) (Fig. 3A); that is, the \( G_e \) increases appeared to be shaped by a tonic increase in the synaptic inputs, whereas the \( G_i \) increases appeared to result from an increase in the number of large IPSC barrages. The mean \( G_e \) and \( G_i \)
values increased to similar degrees in response to flashes (Fig. 3E), whereas an increase in the SD was identified in the Ge, but not in the Gi (Fig. 3F; p = 9.21 × 10−3, t(6) = 3.78, Ge vs Gi, Student’s t test, n = 7 cells from 7 mice).

Spike timing during late responses

In the previous analysis, we demonstrated that, even though the overall balance of excitatory and inhibitory synaptic inputs was maintained during the late response, their input patterns differed for individual trials (Fig. 3A). This finding implied that excitatory and inhibitory inputs may show transient imbalances and thereby determine the timing of late spikes. To characterize the synaptic mechanisms that determine late spike timing, it is important to identify which of the two synaptic inputs exerted more control over the spike timing. We investigated this issue using the dynamic-clamp conductance injection technique. Due to methodological limitations, we were not able to record Ge and Gi simultaneously within a single trial. Therefore, we applied dendrogram clustering to flash-evoked Ge and Gi patterns to classify them into several subsets (Fig. 3G,H). Then, we selected four representative patterns of Ge and Gi (poststimulus 2.5 s in length) from these subsets so that we could maximize the combinatorial repertoires of the Ge and Gi patterns. We subtracted the preflash baseline from individual Ge and Gi patterns (ΔGe and ΔGi, re-
Figure 7. Selective activation of the thalamic reticular nucleus inhibits spikes of dLGN neurons during the time window of V1 late responses. A, AAV2-EP1α-DIO-hChR2(H134R)-EYFP was injected into the thalamic reticular nucleus (TRN) of VGAT-Cre transgenic mice to express ChR2 selectively in GABAergic neurons in the TRN. Confocal images of the ChR2-expressing axons (top) and cell bodies (bottom) of TRN neurons are shown in the insets. Scale bar, 20 μm. B, Schematic of the experimental protocol. Multunit recordings were acquired from the dLGN while a blue light was applied to the dLGN through an optic fiber implant to activate the ChR2-expressing axons of TRN neurons, resulting in the specific inhibition of LGN neurons. C, Spike raster plot of dLGN multunit recordings (top) and its peristimulus time histogram (bottom). Fifty trials of flash stimuli were presented to the contralateral eye without (left, light OFF) or with (right, light ON) blue light stimulation. For the light ON trials, a blue light was presented 0.2–1.2 s after a flash stimulus to inhibit the dLGN specifically in the time window of the V1 late response without affecting the early response. D, Mean ± SEM multunit spike rates of light ON and light OFF trials, respectively. *p = 0.0073, *p = 0.0073, t(6) = 5.02, n = 5 recordings from 2 mice, paired t test.

Source of the V1 late response

Because flash-induced late responses were generated by organized synaptic inputs, they comprised a network-dependent phenomenon. We subsequently searched for the brain region that initiated the late response. Because late responses were observed in both the awake and anesthetized states, we conducted the following experiments mainly using anesthetized animals. We examined two major candidates: top-down feedback pathways from higher-order cortices (Zhang et al., 2014; Manita et al., 2015) and bottom-up feedforward pathways from the retina or thalamus.

We first investigated whether V1 late responses depended on the activity of higher-order cortices. Visual information flows from V1 to the anterior cingulate cortex (ACC), as well as to the secondary visual cortex (V2) (Mohajerani et al., 2013; Zhang et al., 2014). These higher cortical regions also project back to V1 and modulate V1 neuronal activity (Zhang et al., 2014). We evaluated whether the inhibition of ACC activity abolishes the V1 late response. We applied 10 μM tetrodotoxin, an inhibitor of voltage-sensitive sodium channels, to the pial surface of the ACC of anesthetized mice for 20 min. This condition was sufficient to inhibit the neuronal activity of this region (Funayama et al., 2015). We subsequently recorded flash-evoked LFP responses in V1. The application of tetrodotoxin slightly reduced the flash-induced late LFP powers; however, its effect was not statistically significant and the late response was largely intact (data not shown, p > 0.05; paired t test, n = 6 mice). Therefore, we concluded that ACC is not likely the major synaptic driver of the late response, at least under anesthesia.

We subsequently examined the possibility that the late response uses the same feedforward path as the early response. Therefore, we hypothesized that the V1 late response is generated upstream of the visual pathway. We first conducted multunit recordings from the dLGN from anesthetized mice. To permit reliable access to the dLGN, a part of the primary somatosensory cortex and the hippocampus above the dLGN were removed. After this surgical treatment, we conducted routine recordings...
to monitor the dynamics of dLGN neurons (Fig. 5A, B). Similar to the case in V1 neurons, a flash stimulus induced three types of response patterns. Of 36 recordings, six recordings (16.7%) showed early spiking only, one recording (5.6%) showed late spiking only, and 28 recordings (77.8%) showed both early and late spiking (Fig. 6C, D). This response variability may reflect the sum of multiunit spikes from a few neurons in each recording; for instance, a recording that showed both early and late spikes may contain both an early-spiking neuron and a late-spiking neuron or it may contain a neuron that showed both early and late spikes.

Thalamic activity pattern is known to be influenced substantially by brain states such as anesthesia, sleep, and arousal (Sherman, 2001; Weyand et al., 2001). Although we observed late visual responses of V1 neurons in both awake and anesthetized states, it is possible that dLGN neurons exhibit late responses only in anesthetized animals. Therefore, we recorded multiunit recordings from the dLGN in awake mice. Under these conditions, we observed biphasic dLGN activity similar to that under anesthesia (Fig. 5C).

We next examined the $V_m$ responses individual dLGN neurons of awake mice using whole-cell current-clamp recordings. We successfully performed current-clamping on six LGN neurons and the traces of all six cells are presented in Figure 6. The $V_m$ responses of dLGN neurons varied between cells. Some neurons showed either early or late $V_m$ responses (cells 2, 3, 4, and 5), whereas other neurons showed both responses (cells 1 and 6). The $V_m$ waveforms were also variable among cells. Cell 1 reliably showed a sharp late depolarization (cell 1), whereas other neurons showed a large hyperpolarization before late depolarization (cells 2 and 6). Because most V1 neurons exhibited both early and late $V_m$ depolarization, the dLGN responses were different from those of V1 neurons; nonetheless, we confirmed that late-responding neurons were present in the dLGN of awake mice.

Although a flash evoked a late response in the dLGN, it remains possible that V1 received late synaptic inputs via other afferents (Felleman and Van Essen, 1991; Zingg et al., 2014). To confirm the necessity of dLGN late activity for V1 late responses, we investigated whether the timed inhibition of the dLGN activity during the time window of the V1 late response suppresses the V1 late response. If the late response arises through the same pathway as the early response, then the V1 late response would disappear when the dLGN is silenced specifically during the late response. To address this question, we used an optogenetic technique described previously (Reinhold et al., 2015). To silence the relay neurons of the dLGN, we expressed channelrhodopsin-2 (ChR2) in GABAergic neurons of the thalamic reticular nucleus (Hallasa et al., 2011), a region that sends a direct GABAergic projection to the dLGN (Guillery and Harting, 2003; Saalmann and Kastner, 2009). We conditionally expressed ChR2 by injecting a Cre-dependent adeno-associated virus encoding ChR2 into the thalamic reticular nucleus of a VGAT-Cre transgenic mouse (Fig. 7A, B; Vong et al., 2011). The illumination of ChR2-expressing GABAergic axons in the dLGN with a blue light 0.2–3.2 s after the flash-evoked early response suppressed the late dLGN activity ($t = 0.0073, t_{(6)} = 3.57, p = 0.022, t_{(6)} = 3.03$, paired $t$ test).

Figure 8. Selective inhibition of dLGN suppresses the V1 late response. A, A blue light was applied to the dLGN to activate ChR2-expressing axons of TRN neurons. Flash-induced LFP responses were recorded from V1 layer 2/3 without (left) and with a blue light (right, light ON). In the light ON trials, a blue light was presented 0.2–2.2 s after a flash. Top, Stimulus-evoked LFP traces are shown as the mean ± SD. Bottom, Mean powers of flash-evoked LFPs were computed using the wavelet transform. B, Mean powers of LFPs in A were analyzed during the time windows of the early response (left, 0–0.3 s) and the late response (right, 0.4–2.0 s) using fast Fourier transform. C, LFP powers in the gamma-frequency range (30–60 Hz) were compared between the light OFF trials (black) and light ON trials (blue) in all seven animals tested (50 trials each). *$p = 0.011, t_{(6)} = 3.57, p = 0.022, t_{(6)} = 3.03$, paired $t$ test. The effect of blue light continued throughout the recording session and did not decline over sequential trials. Although we targeted GABAergic neurons in the thalamic reticular nucleus, it was also possible that virus spread induced ChR2 expression in a subset of GABAergic neurons in the dLGN; however, such unexpected ectopic expression, if any, did not prevent our intended silencing of relay neurons in the dLGN. Using this technique, we monitored the V1 LFP responses to flash stimuli (Fig. 8A). The specific silencing of the dLGN late response suppressed the V1 late response selectively while sparing the V1 early response (Fig. 8A, B). The late LFP power at each frequency band was reduced significantly to the spontaneous level (Fig. 8C; 4–12 Hz: *$p = 0.025$ light ON vs light OFF, $t_{(6)} = 2.94, t_{(6)} = 0.024$ vs prestimulus baseline spike rate, $t_{(6)} = 2.98$; 14–30 Hz: *$p = 0.022, t_{(6)} = 3.06, p = 0.025, t_{(6)} = 2.98$; 30–60 Hz: *$p = 0.011, t_{(6)} = 3.57, p = 0.022, t_{(6)} = 3.03,$
paired t test, \( n = 7 \) mice). Therefore, the V1 late response depends on late synaptic inputs from the dLGN.

In the feedforward pathway, the dLGN receives driving inputs primarily from retinal ganglion cells. Retinal cells are classified into >50 types of neurons (Enroth-Cugell and Robson, 1966; Boycott and Wasse, 1974; Siegert et al., 2009), some of which may exhibit rebound activity to a brief direct current injection (O’Brien et al., 2002; Mitra and Miller, 2007a, 2007b; Guo et al., 2013). Therefore, we speculated that the retinal rebound spikes may account for the late response identified in the dLGN. We recorded multiunit recordings from the retina in vivo and applied flashes to the same eye (Fig. 9A). The retinal cells responded immediately after the flash with a transient increase in the firing rates (Fig. 9B, C). On average, this early response had a peak spike rate of \( 117.2 \pm 48.8 \) Hz and a peak latency of \( 59.0 \pm 13.4 \) ms (mean ± SD of 5 recordings). However, in contrast to our expectation, the retinal cells did not exhibit apparent late spiking (Fig. 9D, E; \( 3.2 \pm 3.7 \) Hz, \( p = 0.18 \), \( t_{adj} = 1.62 \), paired t test vs the baseline spike rate, \( n = 5 \) recordings from 5 mice). Therefore, the earliest region in which we identified the late response was the dLGN.

Discussion

In this study, we investigated the functional organization of synaptic inputs that constitute the V1 late response. We discovered that the late response was produced by a balanced increase in the \( G_c \) and \( G_o \), whereas the dynamics of the \( G_i \) were more influential in regulating the spike timing of the neuron. The V1 synaptic activation was driven by the thalamo-cortical activity of the dLGN; however, it did not originate in the retina, indicating that the late response has a different mechanism from that of the early response.

Top-down control by higher-order cortices is often reported to modulate the later part of the sensory response (Zhang et al., 2014; Manita et al., 2015). This feedback activity is likely related by sensory perception. In the case of vision, the ACC is one of the neocortical centers that provide top-down modulation of V1 activity (Zhang et al., 2014). We demonstrated that the silencing of the ACC reduced the late LFP power of the flash-evoked response; however, its effect was only partial and a large portion of the late response remained. Therefore, a higher-order cortex may modulate V1 through feedback activity but may not be an essential driver of the late response, at least under conditions of anesthesia.

Similar to V1, the dLGN exhibited flash-induced early and late spiking in both anesthetized and awake states. In addition, time-selective optogenetic silencing of the dLGN, which relays visual information directly to V1, almost completely abolished the V1 late responses. This finding suggested that the generation of V1 late responses requires thalamic activity. Therefore, a component upstream of the classical feedforward pathway such as the dLGN or retina is the most likely candidate for the neural source of the late response. Moreover, retinal cells showed no late spike responses to flash stimuli. Therefore, we speculated that the V1 late response is likely initiated in the dLGN. In the thalamus, there are several types of relay neurons that emit rebound spikes to direct current stimulation (McCormick and Huguenard, 1992). This rebound spiking is generated through hyperpolarization-induced deinactivation of low-threshold calcium channels (Greiner et al., 1998; Timofeev and Steriade, 1998). Moreover, recent studies have demonstrated that the intrathalamic interactions control the activity of sensory relay neurons (Halassa et al., 2014; Wimmer et al., 2015). Because our optogenetic manipulation may also silence the activity of thalamic nuclei other than the dLGN, it is possible that the intrathalamic interactions contribute to the V1 late responses.

Although the dLGN is involved in the generation of late response, the generation process itself is likely to be more complicated. The primary sensory thalamus forms a thalamocortical loop with the primary sensory cortex, in which layer 6 cortical neurons give rise to direct excitatory projections back to the primary sensory thalamus (Mease et al., 2014). In the dLGN, this corticothalamic projection contributes up to 40% of the total synapses, whereas retinal inputs account for only 10% (Van Horn et al., 2000). Therefore, this thalamocortical loop could also initiate and shape flash-induced late responses.

Thalamic nuclei are thought to control higher-order functions such as cognition and perception because they are positioned to modulate the sensory gain to the cortex efficiently (Rees, 2009; Saalmann and Kastner, 2011). Their activity is enhanced during top-down attention in vision (Chalupa et al.,...
interactions are involved. Additional studies are necessary to de-
generate the late response or if intrathalamic or other circuit
thalamocortical component of the late visual response in V1.

suggests that a local cortical circuit maintains the late responses.
Even during the sensory response, the majority of the inputs that reach
a cortical neuron are derived from the cortical recurrent network
(Peters and Payne, 1993). Therefore, the cortical recurrent network
amplifies the thalamic input through reverberation-like
neuronal activation, but the amplification occurs only in the
presence of thalamic activity. Consistent with this idea, the late
response of the dLGN was smaller than the early response (Fig.
5B), whereas in V1, the late response was comparable or even
larger than the early response (Fig. 1B,D,E). Therefore, the late
response is more likely subject to recurrent amplification.
Moreover, both excitatory and inhibitory synaptic inputs occurred in
V1 layer 2/3 neurons during the late response and were balanced
instantaneously on average. This coordinated synaptic balance
suggests that a local cortical circuit maintains the late responses.

To our knowledge, this study is the first to demonstrate a
thalamocortical component of the late visual response in V1.
However, it remains unknown whether thalamic relay neurons
generate the late response or if intrathalamic or other circuit
interactions are involved. Additional studies are necessary to
determine how thalamocortical late inputs interact with feedback
inputs from higher-order cortices.

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