Target-Specific Properties of Thalamocortical Synapses onto Layer 4 of Mouse Primary Visual Cortex

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In primary sensory cortices, thalamocortical (TC) inputs can directly activate excitatory and inhibitory neurons. *In vivo* experiments in the main input layer (L4) of primary visual cortex (V1) have shown that excitatory and inhibitory neurons have different tuning properties. The different functional properties may arise from distinct intrinsic properties of L4 neurons, but could also depend on cell type-specific properties of the synaptic inputs from the lateral geniculate nucleus of the thalamus (LGN) onto L4 neurons. While anatomical studies identified LGN inputs onto both excitatory and inhibitory neurons in V1, their synaptic properties have not been investigated. Here we used an optogenetic approach to selectively activate LGN terminal fields in acute coronal slices containing V1, and recorded monosynaptic currents from excitatory and inhibitory neurons in L4. LGN afferents made monosynaptic connections with pyramidal (Pyr) and fast-spiking (FS) neurons. TC EPSCs on FS neurons were larger and showed steeper short-term depression in response to repetitive stimulation than those on Pyr neurons. LGN inputs onto Pyr and FS neurons also differed in postsynaptic receptor composition and organization of presynaptic release sites. Together, our results demonstrate that LGN input onto L4 neurons in mouse V1 have target-specific presynaptic and postsynaptic properties. Distinct mechanisms of activation of feedforward excitatory and inhibitory neurons in the main input layer of V1 are likely to endow neurons with different response properties to incoming visual stimuli.

Key words: LGN; microcircuitry; optogenetics; synapses; thalamocortical; visual cortex

Introduction

The responsiveness of cortical circuits to sensory stimuli depends on the dynamics of activation of different neuron types. Excitatory and inhibitory neurons differ in their tuning to stimulus features and interocular bias. These differences are particularly marked in layer 4 (L4), the layer that receives the strongest thalamocortical (TC) projection (Cardin et al., 2007; da Costa and Martin, 2011). When compared with pyramidal (Pyr) neurons, inhibitory neurons are generally more broadly tuned to stimulus orientation (Niell and Stryker, 2008; Kuhlman et al., 2011; Zariwala et al., 2011; Atallah et al., 2012; Li et al., 2012; Cottam et al., 2013; Runyan and Sur, 2013), fire at higher frequencies in response to moving grating stimuli (Zhuang et al., 2013), and show less interocular bias (Yazaki-Sugiyama et al., 2009; Kameyama et al., 2010). The mechanisms regulating these differences are incompletely understood. Biophysical properties and firing patterns of excitatory and inhibitory neurons may partly explain their distinct responsiveness (Agmon and Connors, 1992; Contreras and Palmer, 2003). In addition, some of

these differences could be shaped by TC inputs if they showed target-specific properties, as in other sensory cortical circuits.

Recordings *in vivo* strongly suggest that excitatory and fast-spiking (FS) neurons are directly activated by incoming visual inputs (Contreras and Palmer, 2003; Cardin et al., 2007), from retinotopically aligned neurons in the dorsolateral geniculate nucleus of the thalamus (dLGN; Zhuang et al., 2013). Inputs from the dLGN are thought to activate a small number of powerful inputs on L4 neurons (Freund et al., 1985; Stratford et al., 1996). The presynaptic and postsynaptic properties of dLGN inputs on excitatory and inhibitory neurons in L4 of visual cortex (V1) have not been investigated; thus, it is currently unknown whether the distinct response properties of this neuronal population could be due, at least in part, to target-specific dynamics of activation.

Here we confirm that LGN afferents make direct synaptic contacts with excitatory Pyr and FS neurons in L4 of mouse V1. Consistent with previous studies in the primary somatosensory cortex (S1; Cruikshank et al., 2007) and in the primary auditory cortex (A1; Viaene et al., 2011; Schiff and Reyes, 2012), LGN inputs in both Pyr and FS neurons can be classified as type I (or drivers; Sherman, 2007, 2012), as their activation evokes large currents showing short-term depression to repetitive stimulation. LGN inputs in distinct neuron types differ significantly in the number of presynaptic release sites, postsynaptic receptor composition, conductance, and number of postsynaptic channels contributing to the evoked response. Our data suggest that target-specific mechanisms of activation of LGN inputs contribute to the distinct dynamics of activation of excitatory and inhibitory neurons evoked by visual stimuli.

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Materials and Methods

Surgical procedures. The surgery and experimental procedures were approved by the Stony Brook University Animal Use Committee. Both male and female mice were included in the study. To selectively drive LGN terminal fields in V1, we introduced the light-gated conductance channelrhodopsin-2 (ChR2) in the LGN using the vector adeno-associated virus serotype 9 (AAV9; Gu et al., 2012; Wang et al., 2013). The construct was injected into the LGN of postnatal day 15 (P15) mice anesthetized with a cocktail of 100 mg/kg ketamine and 10 mg/kg xylazine using a Nanoject pressure injection system (50×10^{12} viral particles/nl; Wang et al., 2013). The coordinates of injection were 2.0 mm posterior from bregma, 1.9 mm lateral from the midline, and 2.9 mm below the pia. This procedure resulted in the expression of ChR2-GFP localized in the LGN following a 10 d incubation period (Fig. 1A). The construct did not leak into the cortical area above the injection site, so there was no aspecific expression of the construct in the structures above the LGN. The subtype of AAV was chosen for its preferred anterograde transport. To further confirm the lack of retrograde transport, we quantified the possible presence of backfilled somata across the entire cortical mantle in each slice used for this study. None of the preparations showed backfilled somata in any layer of V1, similar to what we reported in our previous study (Wang et al., 2013). To assess the reliability of the levels of expression of the ChR2-GFP construct in the LGN and in LGN terminal fields in V1 across preparations, a group of animals was perfused transcardially with 4% paraformaldehyde in phosphate buffer 10 d after injection. Coronal slices containing either LGN (to verify the location of the injection site) or V1 (100 μ m) were visualized with confocal microscopy. The location, spread, and intensity of the GFP signal was quantified using ImageJ. In slices containing V1, the intensity profile of the GFP signal was quantified in regions of interest (ROIs) spanning the cortical mantle (ROI: 40 μ m wide and extending from the pial surface to the border between layer 6 and the white matter). A calibration curve of the levels of GFP fluorescence across layers was calculated as the average $\pm$ SD of the GFP intensity measured in 15 slices from six animals (Fig. 1C, green line, average, black lines, $\pm$ SD). The intensity of the GFP signal of all subsequent preparations was compared against this curve. Recordings were excluded from the analysis if obtained from slices in which the GFP signal deviated more than 1 SD from the calibration curve.

Recordings. Mice were deeply anesthetized by placing them in a closed jar saturated with isoflurane vapors. They were then decapitated, and the brain was dissected out to prepare acute coronal slices containing LGN or V1 10 d after injection of the AAV9–ChR2–GFP construct (Wang et al., 2013). For each preparation, the location of the injection site was visually identified on the day of the experiment and histologically verified *post hoc*. The functional expression of the construct was assessed by preparing acute LGN slices and recording from LGN neurons. Brief (2 ms) light stimuli (470 nm) were delivered through a 40 $\times$ water-immersion objective of an upright microscope (BX51WI, Olympus) positioned directly

Figure 1. Expression of ChR2-GFP in LGN and V1. **A**, Left, Representative histology of a section showing expression of ChR2-GFP at P27 (injection at P15). Green, ChR2-GFP; blue, DAPI. Scale bar, 1 mm. Right, Enlarged image of the region indicated by the white square in the image on the left. Scale bar, 40 μ m. **B**, Top, Slice of LGN expressing ChR2-GFP and containing a recorded neuron (indicated by the arrowhead). The somatic expression of the injected construct (green) is colocalized with the biocytin staining (red), thus the recorded neuron appears yellow. Scale bar, 40 μ m. Bottom, Light-evoked action potentials in the LGN neuron shown in the top image. Light stimulus intensity, 0.3 mW/mm². Trains of light pulses at 5, 10, and 20 Hz were delivered while the neuron was recorded in the presence of AMPA and NMDA receptor blockers to ensure that the action potentials depended on the activation of the light-gated protein. **C**, Left, Expression of ChR2-GFP in the LGN terminal fields in V1. Right, Calibration curve of GFP intensity across the cortical mantle (see Materials and Methods). Green, Average GFP intensity across 15 slices from six animals; black traces, $\pm$ 1 SD from the average fluorescence calculated across the 15 slices. Scale bar, 100 μ m.

above the recording chamber, and their ability to reliably elicit action potentials in LGN neurons was determined. This procedure was performed at the beginning of each experiment. To further test that action potentials in LGN neurons were driven by light pulses and not by synaptic activity, the experiments were repeated in the presence of 20 μ M 6,7-dinitroquinoxaline-2,3(1*H*,4*H*)-dione (DNQX) and 50 μ M D-2-amino-5-phosphonovaleate (APV) to block AMPA receptors (AMPA) and NMDA receptors (NMDARs; Fig. 1B).

After verification that the expression level of the construct in the LGN was sufficient to drive LGN neurons above the threshold for action potentials (Fig. 1B), recordings were performed in acute coronal slices containing V1, where light stimuli were used to selectively activate the LGN

terminal fields. Different neuron types in L4 were visually identified under infrared microscopy. In these experiments, LGN terminal fields were driven with either one or multiple 2 ms pulses of 470 nm light. Light intensity was regulated through an epifluorescent light source (range of intensity at the tissue level, 0.1–0.3 mW/mm²), and passed through a blue filter. Light stimulation was synchronized with electrophysiological recordings via the connection of a shutter (Sutter) to the analog output of a Multi-patch-clamp amplifier (HEKA). The power of the light stimuli in the recording chamber was quantified using an optical power meter (Coherent, Inc). Light-evoked synaptic responses were aligned off-line at 10–90% of rise time. Four simultaneous patch-clamp recordings were used to compare the properties of LGN-evoked responses in distinct neuron types within the same slice before quantifying population data across preparations.

Criteria to define responses as monosynaptic. TC EPSCs were considered monosynaptic if their delay from stimulus onset was <2.5 ms and did not show any additional peak on the decay phase of the postsynaptic current. A group of Pyr (17%) and FS (34%) neurons showed disynaptic currents, which were detected as additional peaks on the decay phase of the evoked response. The delay from stimulus onset of those later peaks was on average 11.4 ± 0.2 ms. As the theoretical reversal potential in our internal solution is -50 mV, even inhibitory disynaptic events are detectable as inward currents on the decay phase of the evoked TC EPSC. All recorded neurons showing disynaptic responses were excluded from the analysis; therefore, the number of samples reported in the manuscript selectively indicates neurons whose responses fit our definition of monosynaptic TC EPSC. Nonstationary peak-scaled noise analysis and analysis of release properties were performed on TC EPSCs evoked with minimal light intensity to activate putative single axons. While minimal light intensity evoked TC EPSCs with amplitudes comparable to those evoked by focal laser stimulation (Cruikshank et al., 2010) and those evoked by minimal electrical stimulation in thalamocortical slices in the somatosensory cortex (Beierlein et al., 2003) in both Pyr and FS neurons, in our preparations we did not observe failures. The absence of failures likely depends on the depolarization of terminals evoked by direct activation of the light-gated sodium conductance expressed by LGN axons.

Post hoc neuron identification. During recordings, neurons were filled with 0.4% biocytin. After completion of the recordings, slices were fixed in a 4% paraformaldehyde PBS solution for 1 week. Slices were then washed in PBS, permeabilized with 1% Triton X-100 in PBS for 2 h, and incubated overnight at 4°C in a solution containing streptavidin-conjugated AF-594 (1:1000, Invitrogen) and 0.1% Triton X-100 in PBS. After this incubation period, slices were washed in PBS, mounted on glass slides with Fluoromount, and coverslipped. Slices were imaged with a fluorescent microscope (Axioskop, Zeiss). Only neurons located in L4 were used in the analysis.

Nonstationary noise analysis. Nonstationary noise analysis (Sigworth, 1980; Traynelis and Jaramillo, 1998; Hartveit and Veruki, 2007) was applied to determine the single-channel conductance and number of open channels of EPSCs (i.e., TC EPSCs). The analysis was performed on TC EPSCs recorded at -70 mV in the presence of the NMDA receptor blocker APV ($50 \mu\text{M}$) to determine differences in the properties of the AMPA receptor-mediated component of the TC EPSC. For each neuron, 10 light-evoked TC EPSCs were averaged, and each individual trace was scaled to the mean. The variance of the TC EPSCs during the decay phase was averaged in 1 ms bins, and plotted against the mean current. This plot was fitted to a polynomial equation $\sigma^2 = iI - (I^2/N)$ to calculate unitary current (i_u) and the number of open channels (N_o).

Analysis of release properties. Strontium chloride (SrCl_2) is known to produce delayed asynchronous release (Goda and Stevens, 1994) and has a long lifetime in the presynaptic terminal (Xu-Friedman and Regehr, 2000). Bath application of SrCl_2 was shown to decrease the amplitude of an evoked response down to that driven by release at a single release site (Morishita and Alger, 1997; Bartley et al., 2008). The amplitude of a single release site-evoked response varies depending on the presynaptic cell (Daw et al., 2009). The remaining desynchronized release sites produce synaptic events that can be detected in the decay phase of the first evoked response (up to 200 ms from stimulus onset). In a set of experiments, $10 \text{ mM } \text{SrCl}_2/0 \text{ mM } \text{CaCl}_2$ was bath applied following the acquisition

of a 10 min baseline TC EPSC. The effect of SrCl_2 on the amplitude of the evoked TC EPSC was quantified, and the number of subsequently activated release sites was inferred by assessing the number of events detected across the 200 ms time window from the light stimulus.

Quantal analysis. To assess the site of action of SrCl_2 , quantal analysis was performed on the first evoked response before and after application of SrCl_2 (Sola et al., 2004; Wang et al., 2012). A binomial model (Wernig, 1975) was fit to the data to calculate the probability of release (p) and the number of release sites (n) from the average TC EPSC amplitude and coefficient of variation (CV). Statistical comparisons of CVs before and after applications of SrCl_2 were also provided for each neuron type.

Statistical analysis. All data are presented as the mean $\pm$ SE. Statistical significance was determined using two-tailed unpaired Student's t tests. Significant differences across groups were assessed with one-way ANOVAs. Spearman rank-order correlation analysis was applied to determine the possible correlations of the magnitude of the effect of SrCl_2 on the first evoked TC EPSC with the baseline amplitude of the TC EPSC; p values ≤ 0.05 were considered to be significant.

Solutions. Artificial CSF (ACSF) contained the following (in mM): 126 NaCl, 3 KCl, 2 MgSO_4 , 1 NaHPO_4 , 25 NaHCO_3 , 2 CaCl_2 , and 14 dextrose. The internal solution contained the following (in mM): 20 KCl, 100 K-Glu, 10 K-HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-phosphocreatine, and 0.4% biocytin. The pH of the internal solution was adjusted to 7.35 with KOH, and the osmolarity was adjusted to 295 mOsm with sucrose. In all pharmacological experiments, drugs were bath applied. In experiments designed to quantify the NMDA/AMPA ratio, $3 \mu\text{M}$ QX-314 (Tocris Bioscience) was added to the internal solution. All other drugs were bath applied. For isolating AMPA and NMDA receptor-mediated components, $20 \mu\text{M}$ DNQX (Tocris Bioscience) and $50 \mu\text{M}$ APV (Tocris Bioscience) were applied sequentially and additively. To desynchronize release sites, $10 \text{ mM } \text{SrCl}_2$ (Sigma-Aldrich) was added to a CaCl_2 -free ACSF following acquisition of a 10 min baseline.

Results

Selective activation of thalamocortical afferents in acute V1 slices

Obtaining a reliable preparation for the detailed study of the synaptic properties of LGN inputs to V1 has proven difficult due to the complex anatomy of the LGN projection (MacLean et al., 2006). Thus, to selectively activate LGN inputs we used an optogenetic approach that allowed for the stimulation of LGN terminal fields in V1 (Wang et al., 2013). After a 10 d incubation period, LGN slices were prepared to assess the effectiveness of the expression of the ChR2-GFP construct. Whole-cell current-clamp recordings confirmed that LGN neurons expressed the construct and could be driven above the action potential threshold using brief light stimuli (2 ms, 0.3 mW/mm^2), even in the presence of AMPA and NMDA receptor blockers (Fig. 1B). The ability of LGN neurons to fire action potentials following the frequency of stimulation was assessed experimentally. In the presence of AMPA and NMDA receptor blockers, LGN neurons fired action potentials reliably in response to trains of light pulses up to 20 Hz (Fig. 1B). This is consistent with the reported kinetics of activation and deactivation of ChR2 (Zhang et al., 2006).

Acute coronal slices containing V1 were also prepared to visualize and activate LGN terminal fields. To assess consistency of expression across preparations, a fluorescence intensity profile was measured across laminae and a calibration curve for the intensity of GFP fluorescence was obtained by averaging 15 slices from 6 animals that received the AAV9-ChR2-GFP injection in the LGN (Fig. 1C; see also Materials and Methods; Wang et al., 2013). GFP expression for all preparations was then compared against a calibration curve, and only data recorded from slices whose fluorescence profile fell within 1 SD of the mean calibration curve were included in the analysis. The possible presence of

backfilled somata in every layer of V1 was assessed for each preparation. No back-filled somata were observed in any layer, confirming that LGN afferents were the only light-sensitive component of V1 in our preparation.

LGN inputs have cell type-specific properties in layer 4 of V1

Anatomical evidence suggests that the LGN directly innervates both pyramidal and inhibitory neurons in L4 (Freund et al., 1985; Ahmed et al., 1994, 1997; Erisir and Dreusicke, 2005), but whether LGN inputs onto distinct neuron types differ in their synaptic properties has not been investigated. To address this, we recorded from Pyr neurons and two distinct populations of inhibitory neurons, and compared their synaptic responses to the activation of LGN terminal fields. Light activation of LGN axons elicited reliable TC EPSCs in every Pyr (Fig. 2*A,D*) and FS (Fig. 2*B,D*) neuron recorded. In a subset (three of six) of regular spiking nonpyramidal (RSNP) neurons, a small but reliable current was also detected (Fig. 2*C,D*). When the latency from stimulus onset of postsynaptic responses was quantified and compared across cell types, only responses from Pyr and FS neurons were consistent with monosynaptic inputs, while responses in the RSNP neurons were not (Fig. 2*E*; Pyr neurons: 1.62 ± 0.05 ms; $n = 29$; FS neurons: 1.34 ± 0.1 ms; $n = 15$; RSNP neurons: 7.34 ± 0.2 ms; $n = 6$). The three distinct neuron types were often recorded simultaneously using quadruple patch clamp; thus, the differences observed are not due to artifacts of light stimulation or inconsistencies across preparations. Together, these results support the interpretation that Pyr and FS neurons receive strong and reliable LGN inputs with delay from stimulus compatible with monosynaptic connections, while RSNP neurons do not.

Because they receive reliable LGN inputs compatible with the presence of monosynaptic connections, we focused our analysis on the comparison of synaptic properties and dynamics of TC responses evoked in Pyr and FS neurons. TC EPSCs recorded from Pyr neurons had significantly slower rise and decay time constants than those recorded from FS neurons (rise time: Pyr neurons, 1.76 ± 0.08 ms, $n = 29$; FS neurons, 0.9 ± 0.09 ms, $n = 15$; unpaired t test, $p < 0.001$; decay time constant: Pyr neurons, 12.1 ± 0.9 ms; FS neurons, 6.4 ± 0.4 ms; $p < 0.001$). Stimuli with increasing light intensity (0.1 – 0.3 mW/mm²) were used to examine the input/output relationship of LGN inputs in Pyr and FS neurons. The amplitude

Figure 2. Monosynaptic inputs from the LGN into Pyr and FS neurons. **A**, Left, *Post hoc* reconstruction of an L4 pyramidal neuron (green, ChR2-GFP; red, biocytin-Alexa Fluor 594 as in **B** and **C**, left panels). Middle, Firing pattern of the Pyr neuron on the left in response to a 0.2 nA, 1 s current step. Right, Light-evoked TC EPSCs recorded from the neuron shown in the left panel (light pulse: 2 ms, 0.3 mW/mm²; same for the right panels in **B** and **C**, and for **D**). Gray, Single traces; black, average of 30 traces. **B**, Left, FS neurons recorded in L4 of the V1. Middle, Firing pattern of the FS neuron on the left in response to 0.3 nA, 1 s long current step. Right, Light-evoked TC EPSC recorded from the FS neuron on the left. Gray, Single traces; black, average of 30 traces. **C**, Left, *Post hoc* reconstruction of an RSNP neuron recorded in L4. Middle, Firing pattern of the L4 RSNP shown on the left in response to a 0.2 nA current pulse. Right, TC EPSC evoked by a brief light pulse and recorded from the RSNP neuron on the left. Gray, Single traces; black, average of 30 traces. **D**, Time course of light-evoked TC EPSCs for neurons shown in **A** (black), **B** (gray), and **C** (white), indicating that responses were stable over time. **E**, Average TC EPSC latency from stimulus onset for Pyr, FS, and RSNP neurons. Data are presented as the mean $\pm$ SE. Asterisks indicate significant differences.

of TC EPSCs in FS neurons was significantly larger than those in Pyr neurons for all stimulus intensities except for the lowest (Fig. 3*A–C*; 0.1 mW/mm²: Pyr neurons, 160 ± 39.3 pA; FS neurons, 236.4 ± 132.7 ; $p = 0.07$; 0.15 mW/mm²: Pyr neurons, $217.04 \pm$

Figure 3. Distinct input–output curves and short-term dynamics of TC EPSCs in FS and Pyr neurons. **A**, Sample TC EPSCs recorded from L4 Pyr neurons, in response to a train of five light pulses at 10 Hz. Intensity of light pulses, 0.3 mW/mm². **B**, Sample TC EPSCs recorded from L4 FS neurons in response to a train of five light pulses at 10 Hz. Intensity of light pulses, 0.3 mW/mm². **C**, Input–output curves for L4 Pyr (black) and FS (gray) neurons. **D**, Normalized amplitude of TC EPSCs in the train for Pyr (black) and FS (gray) neurons. Data are presented as the mean $\pm$ SE. Asterisks indicate significant differences.

42.9 pA; FS neurons, 541.9 ± 134.8 pA; $p < 0.02$; 0.2 mW/mm²: Pyr neurons, 269.1 ± 37.8 pA; FS neurons, 680.1 ± 154.5 pA; $p < 0.03$; 0.3 mW/mm²: Pyr neurons, 328.3 ± 41.9 pA; FS neurons, 1009.56 ± 169.2 ; $p < 0.05$; Pyr neurons, $n = 29$; FS neurons, $n = 15$). Furthermore, the slope of the input–output curve was steeper for FS neurons than for Pyr neurons (Pyr neurons, 0.83 nA/mW; FS neurons, 2.91 nA/mW; $p < 0.05$). These results suggest that a single incoming stimulus would likely activate the feedforward inhibitory circuit more effectively than the excitatory circuit.

We next examined the short-term dynamics of TC EPSCs to assess how trains of stimuli may engage feedforward excitatory and inhibitory circuits. We chose to use a 10 Hz frequency of repetitive stimulation, because this frequency is reliably followed by the kinetics of activation and inactivation of ChR2 (Zhang et al., 2006; Gu et al., 2012; Wang et al., 2013), and is reliably followed by LGN neurons (Fig. 1B). In response to trains of five light pulses, we observed short-term depression of TC EPSCs of different magnitude depending on the postsynaptic target. TC EPSCs onto FS neurons showed a larger initial TC EPSC followed by much smaller subsequent events. Pyramidal neurons showed a less dramatic decrease in amplitude of successive TC EPSCs (Fig. 3D). These distinct short-term dynamics resulted in significantly different paired-pulse ratios (PPRs) and steady-state ratios (SSRs) between neuron types (PPRs: Pyr neurons, 0.66 ± 0.04 ; FS neurons, 0.56 ± 0.05 ; $p < 0.01$; SSRs: Pyr neurons, 0.38 ± 0.03 ; FS neurons, 0.25 ± 0.03 ; $p < 0.006$; Pyr neurons, $n = 29$; FS neurons, $n = 15$). Thus, LGN inputs in Pyr and FS neurons differ in both synaptic strength and short-term dynamics.

Before analyzing the specific synaptic mechanisms determining TC EPSC cell type-specific properties, we assessed how repetitive activation of TC terminal fields may affect the ability to fire action potentials in Pyr and FS neurons. To do that, Pyr and FS

neurons were recorded in current clamp. Pyr and FS neurons showed detectable TC EPSPs at all stimulus intensities (Fig. 4A,B). However, every recorded FS neuron fired at least one action potential at both the minimum and maximum light intensity (Fig. 4C, 14 of 14). Conversely, 23.8% (Fig. 4C) of Pyr neurons (15 of 63 neurons; Pearson χ^2 test, $p < 0.02$) fired an action potential at the lowest light intensity, and only 44% (Fig. 4C; 28 of 63 neurons; Pearson χ^2 test, $p < 0.05$) fired at least one action potential at the highest light intensity. The ratio of action potentials per light stimulus was used to calculate a spike reliability ratio (i.e., the SRR). At all stimulus intensities, FS neurons fired significantly more action potentials per light stimulus than pyramidal neurons (Fig. 4D, SRR; 0.1 mW/mm²: Pyr neurons, 0.09 ± 0.03 ; FS neurons, 0.62 ± 0.16 ; unpaired t test, $p < 0.03$; 0.15 mW/mm²: Pyr neurons, 0.1 ± 0.03 ; FS neurons, 1 ± 0.001 ; $p < 0.001$; 0.2 mW/mm²: Pyr neurons, 0.16 ± 0.04 ; FS neurons, 1.56 ± 0.2 ; $p < 0.001$; 0.3 mW/mm²: Pyr neurons, 0.3 ± 0.05 ; FS neurons, 1.7 ± 0.2 ; $p < 0.001$; Pyr neurons, $n = 63$; FS neurons, $n = 14$).

The difference in the ability of LGN inputs to drive Pyr and FS neurons above threshold did not depend on differences in resting input resistance, as FS input resistance (R_i) was lower than that of Pyr neurons (R_i : Pyr neurons, 192 ± 10 M Ω ; FS neurons, 138 ± 8 M Ω ; unpaired t test, $p < 0.02$). We hypothesized that the ability of Pyr neurons to fire action potentials in response to LGN terminal field activation may be shunted by feedforward inhibition. Consistent with this, partial blockade of GABA_A receptors with bath application of picrotoxin (10 μ M) allowed all recorded Pyr neurons to fire action potentials in response to a single light pulse at 0.1 mW/mm² (Fig. 4A, gray overlay; 10 of 10 Pyr). These data indicate that TC afferents drive feedforward inhibition in V1 more effectively than feedforward excitation, and that feedforward inhibition acts as a brake on LGN activation of V1 pyramidal neurons.

Cell type-specific postsynaptic differences of TC EPSCs in V1

Our findings so far suggest that LGN inputs onto Pyr and FS neurons in L4 of the V1 show differences in kinetics, strength, and short-term dynamics. The differences in decay kinetics and average amplitude suggest that these inputs may be driving different receptor types on Pyr and FS neurons. To assess the contribution of different glutamatergic receptors to the TC EPSCs recorded from Pyr and FS neurons, we sequentially and additively applied blockers for AMPA and NMDA receptors (20 μ M DNQX and 50 μ M APV). For these experiments, the TC terminal fields were stimulated using minimal light intensity to limit the contribution of the recurrent circuit to the evoked response. Neurons were recorded at -70 mV to isolate the component of the TC EPSC driven by AMPARs, and at $+40$ mV to isolate the NMDAR component. After acquisition of a 10 min baseline in regular ACSF, 20 μ M DNQX was bath applied for 10–15 min. After that, a mix of 20 μ M DNQX and 50 μ M APV was perfused. The AMPAR- and NMDAR-mediated currents were then isolated and analyzed fol-

lowing off-line subtraction (Fig. 5*A,B*). TC EPSCs in Pyr neurons comprised both an NMDAR- and an AMPAR-mediated component (Fig. 5*C*; NMDAR-mediated component, 54.9 ± 10.3 pA; AMPAR-mediated component, 244.2 ± 45.7 pA; $n = 10$). The NMDAR component accounted for about one third of the TC EPSC (Fig. 5*D*; NMDA/AMPA ratio: 0.27 ± 0.06 , $n = 10$). The AMPAR-mediated current showed no rectification at +40 mV, indicating that TC EPSCs in L4 Pyr neurons contain the AMPAR subunit GluA2 (Boulter et al., 1990; Verdoorn et al., 1991; Jonas and Burnashev, 1995). Differently, bath application of 20 μ M DNQX completely blocked the TC EPSC in FS neurons, indicating that NMDARs did not contribute to the TC EPSCs in FS neurons (Fig. 5*C*; AMPARs: 554.6 ± 90.7 pA, $n = 5$). No AMPAR- and NMDAR-dependent components were detected at +40 mV, indicating that TC EPSCs in FS neurons are mediated by Ca^{2+} -permeable GluA2-lacking AMPARs (Bowie and Mayer, 1995; Geiger et al., 1995) and lack NMDARs (Fig. 5*D*; NMDA/AMPA ratio for FS neurons = 0). All FS neurons were recorded simultaneously with Pyr neurons, thus the differences observed cannot be ascribed to possible inconsistencies in the experimental preparation.

We further analyzed TC EPSCs to identify possible additional mechanisms contributing to the differences in the AMPAR-mediated component of TC EPSCs in Pyr and FS neurons. We used peak-scaled nonstationary noise analysis of TC EPSCs recorded at -70 mV in the presence of 50 μ M APV and evoked by minimal light intensity (0.1 mW/mm^2) to assess possible differences in single-channel conductance and the number of open channels contributing to the AMPA current of the TC EPSC (Sigworth, 1980; Traynelis and Jaramillo, 1998; Hartveit and Veruki, 2007). The peaks of 10 evoked TC EPSCs were scaled to the average peak amplitude (Fig. 6*A,B*), and the variance (σ^2) during the decay phase was measured and plotted against the average amplitude (Fig. 6*C,D*). The plots were fitted with a parabolic curve (Fig. 6*C,D*, equation inset), and values for i_u and N_o were estimated from the equation. This analysis showed that TC EPSCs in Pyr neurons were mediated by a large number of open channels passing a small unitary current (Fig. 6*E*; Pyr neurons: N_o , 101.9 ± 21.9 ; i_u , 4.01 ± 1.48 pA; $n = 11$); differently, TC EPSCs in FS neurons were mediated by a small number of open channels with large unitary current (Fig. 6*E*; FS neurons: N_o , 22.7 ± 3.6 ; i_u , 26.2 ± 6.1 pA; $n = 6$). Statistical comparisons of the number of open channels and unitary currents between cell types indicated significant differences (unpaired t test: N_o , $p < 0.02$; i_u , $p < 0.02$). Together, these data demonstrate that differences in receptor composition, number of open channels, and single-channel conductance explain part of the cell type-specific properties of TC EPSCs in L4 of the V1.

Figure 4. Feedforward inhibition prevents Pyr neurons from firing action potentials. **A**, Top, TC EPSCs recorded from a Pyr neuron in current clamp (light pulse intensity, 0.1 mW/mm^2) in the absence (black) and in the presence of picrotoxin ($10 \mu\text{M}$; gray). Bottom, TC EPSCs recorded from a Pyr neuron in response to light pulses with the highest stimulus intensity (0.3 mW/mm^2). **B**, Top, TC EPSCs recorded from an FS neuron in current clamp at the lowest stimulus intensity (0.1 mW/mm^2). Bottom, TC EPSCs from an FS neuron evoked using the highest stimulus intensity (0.3 mW/mm^2). **C**, Percentage of Pyr (black) and FS (gray) neurons that fired at least one action potential in response to light stimuli at the minimum (0.1 mW/mm^2) and maximum (0.3 mW/mm^2) light intensity. **D**, Spike reliability ratio, defined as the number of action potentials per light pulse, quantified for Pyr (black) and FS (gray) neurons as a function of the stimulus intensity. Data are presented as the mean $\pm$ SE. Asterisks indicate significance.

Cell type-specific presynaptic properties of LGN inputs in L4 of V1

TC EPSCs in L4 of the V1 show cell type-specific short-term depression. This suggests that presynaptic mechanisms may also contribute to differentiate these inputs (O'Donovan and Rinzel, 1997; Stevens and Wesseling, 1998). To compare presynaptic properties, we recorded TC EPSCs in Pyr and FS neurons in the presence of SrCl_2 (10 mM). SrCl_2 desynchronizes release sites (Goda and Stevens, 1994; Bartley et al., 2008) and can be used to assess the number of release sites of a specific synapse (Gil et al., 1999; Daw et al., 2009; Hull et al., 2009). Following bath application of SrCl_2 , there was a significant reduction of TC EPSC amplitude in both Pyr and FS neurons (Fig. 7*A–C*; Pyr neurons: baseline, 408.5 ± 62.2 pA; SrCl_2 , 332.2 ± 47.7 pA; FS neurons: baseline, 675.8 ± 102.9 pA; SrCl_2 , 505.03 ± 89.05 pA; unpaired t test, $p < 0.04$; Pyr neurons, $n = 17$; FS neurons, $n = 6$). The magnitude of the decrease was significantly larger for TC EPSCs in FS neurons than in Pyr neurons (one-way ANOVA, $p < 0.03$). SrCl_2 application did not affect response latency (Pyr neurons: baseline, 2.54 ± 0.09 ms; SrCl_2 , 2.65 ± 0.09 ms; unpaired t test, $p = 0.5$; FS neurons: baseline, 1.93 ± 0.17 ms; SrCl_2 , 2.16 ± 0.07 ms; $p = 0.12$) or rise time (Pyr neurons: baseline, 1.9 ± 0.12 ms; SrCl_2 , 2.1 ± 0.15 ms; $p = 0.2$; FS neurons: baseline, 0.96 ± 0.16

Figure 5. Different postsynaptic receptor contributions to TC EPSCs in Pyr and FS neurons. **A**, TC EPSCs (light intensity, 0.1 mW/mm^2) recorded at -70 mV and $+40 \text{ mV}$ to isolate baseline (left), AMPAR-mediated (middle), and NMDAR-mediated (right) currents in a Pyr neuron. **B**, TC EPSCs (light intensity, 0.1 mW/mm^2) recorded at -70 mV and $+40 \text{ mV}$ to isolate baseline (left), AMPAR-mediated (middle), and NMDAR-mediated (right) currents in an FS neuron. **C**, Average amplitude of NMDAR- and AMPAR-mediated currents from Pyr (black) and FS (gray) neurons. Data are presented as the mean $\pm$ SE. **D**, NMDA/AMPA current ratio for Pyr (black) and FS (gray) neurons. Circles show individual ratios; the black bar shows the mean $\pm$ SE. Asterisks indicate significant differences.

ms; SrCl_2 , 1.03 ± 0.17 ; unpaired t test, $p = 0.23$). Application of SrCl_2 also led to prolonged TC EPSCs, with multiple events becoming detectable during the decay phase. When the distribution of the number of events on the tail of TC EPSCs was quantified and compared between Pyr and FS neurons, we observed that a significantly larger number of events was detectable in FS neurons (Fig. 7D). The larger amplitude decrease and number of events following SrCl_2 application strongly suggest that TC EPSCs in FS neurons depend on the activation of a larger number of release sites compared with those activated in Pyr neurons.

To further understand the presynaptic mechanisms that differentiate LGN inputs in L4 Pyr and FS neurons, we compared the CV of TC EPSCs before and after bath application of SrCl_2 . The baseline CV of TC EPSCs in both Pyr and FS neurons was already quite low, suggesting that LGN inputs in both neuron types have a high release probability. However, the CV of TC EPSCs in FS neurons was significantly lower than that in Pyr neurons (Fig. 7E; baseline CV: Pyr neurons, 0.12 ± 0.01 ; FS neurons, 0.07 ± 0.01 ; unpaired t test, $p < 0.006$; Pyr neurons, $n = 17$; FS neurons, $n = 6$). This indicates that LGN inputs in FS neurons likely have a higher release probability than those in Pyr neurons in response to a single stimulus. After SrCl_2 application, the CV of TC EPSCs in Pyr neurons decreased significantly (Fig. 7E, SrCl_2 ; Pyr neurons, 0.07 ± 0.01 ; paired t test vs baseline CV, $p < 0.01$), suggesting that TC EPSCs in Pyr neurons are mediated by the activation of a small number of inputs composed of a few, highly reliable release sites (Freund et al., 1985; Stratford et al., 1996).

Although the amplitude of TC EPSCs in FS neurons was significantly reduced by SrCl_2 , their CV remained unaltered (Fig. 7E, SrCl_2 ; FS neurons, 0.067 ± 0.005), suggesting that this input is mediated by clusters of highly reliable release sites (Bagnall et al., 2011).

Quantal analysis of TC EPSCs before and after SrCl_2 application was performed to further determine what mechanisms could account for the decrease in amplitude observed in TC EPSCs in FS neurons: release probability (p), quantal size (q), or number of release sites (n ; Sola et al., 2004). As shown in Figure 7F, all of the data point to TC EPSCs in FS neurons falling on or close to the unity line, suggesting that SrCl_2 application affected the number of release sites at the LGN–FS synapse the most. As for Pyr neurons, the single data points were more scattered, although their average value was also positioned on the unity line. This suggests that SrCl_2 application at the LGN–Pyr neuron synapse affects the number of release sites simultaneously activated by the light stimulus, but that additional effects of SrCl_2 on release probability may also be involved.

Spearman rank order correlation analysis was used to determine whether the magnitude of the effect of SrCl_2 was correlated with the baseline amplitude of TC EPSCs. Pyr neurons showed no significant correlation ($R = 0.39$, $p = 0.11$), consistent with the small effect or lack of effect of SrCl_2 on the amplitude of TC EPSC on most Pyr neurons (14 of 17 neurons) recorded (Fig. 7G; $R = 0.15$; $n = 17$). Differently, FS neurons showed a significant correlation between the initial TC EPSC amplitude and the magnitude of the effect of SrCl_2 (Fig. 7H; $R = 0.83$; $p < 0.05$; $n = 6$). These results support the interpretation that the amplitude of the TC EPSC in FS neurons depends on the number of release sites located at the LGN terminals in FS synapse. All FS neurons were recorded simultaneously with pyramidal neurons, and only the lowest stimulus intensity was used for these experiments (0.1 mW/mm^2) to minimize the contribution of recurrent intracortical circuits. Our results indicate that LGN inputs in Pyr and FS neurons rely on target-specific presynaptic and postsynaptic mechanisms. Thus, differences in LGN activation of Pyr and FS neurons may account, at least in part, for their distinct functional properties.

Discussion

We have analyzed the properties of LGN synapses in Pyr and FS neurons in L4 in the V1. LGN inputs in Pyr and FS neurons presented some similarities—they were powerful and reliable, and showed short-term depression in response to repetitive stimulation—but relied on distinct presynaptic and postsynaptic mechanisms. A larger proportion of FS neurons reliably fired action potentials at all stimulation intensities, indicating that single incoming stimuli likely activate feedforward inhibitory circuitry more effectively than excitatory circuits. This is consistent with previous findings showing that *in vivo* putative inhibitory neurons show higher activity evoked by visual stimuli than excitatory neurons (Zhuang et al., 2013). TC EPSCs in FS neurons were larger and depressed more in response to repetitive stimulation than TC EPSCs in Pyr neurons, suggesting that stimuli containing trains of spikes could successfully overcome feedforward inhibition and drive excitatory neurons.

TC EPSCs in Pyr and FS neurons showed target-specific presynaptic and postsynaptic properties. LGN synapses in Pyr neurons activated both NMDARs and AMPARs. The AMPAR-mediated current had similar amplitudes at positive and negative potentials, indicating a contribution of calcium-impermeable AMPARs (Boulter et al., 1990; Verdoorn et al., 1991; Jonas and

Burnashev, 1995). The NMDAR-mediated current was large at -70 mV, suggesting that these receptors contain Mg^{2+} -insensitive subunits and contribute to evoked responses at resting membrane potential (Fleiderovich et al., 1998; Binshtok et al., 2006). LGN synapses in FS neurons lacked NMDAR-mediated currents and contained inwardly rectifying AMPAR-mediated currents, indicating that these receptors lack the GluA2 subunit and are likely calcium permeable (Bowie and Mayer, 1995; Geiger et al., 1995). Peak-scaled nonstationary noise analysis of the AMPAR-mediated current showed that TC EPSCs in Pyr neurons resulted from the activation of many open channels with small unitary current, consistent with the presence of GluA2-containing AMPA receptors (Swanson et al., 1997). Differently, TC EPSCs in FS neurons depend on a small number of open channels with large unitary current, consistent with the presence of GluA2-lacking AMPARs (Hestrin, 1993).

The differences in short-term dynamics and the effects of strontium chloride on TC EPSCs indicate that LGN inputs also have target-specific presynaptic properties. Strontium chloride induced small, although significant, changes in the amplitude of TC EPSCs in Pyr neurons. Few quantal events were detected after the first evoked response, suggesting that LGN synapses in Pyr neurons contain a small number of powerful release sites. This is consistent with the shallow slope of the input–output curve for TC EPSCs in Pyr neurons, their low coefficient of variation, and with previous reports that single LGN axons make a small number of synaptic contacts with L4 excitatory neurons in the V1 (Freund et al., 1985; Peters and Payne, 1993; da Costa and Martin, 2009). The amplitude of TC EPSCs in FS neurons was reduced by one third to one half by the application of strontium chloride. Up to 20 quantal events could be detected in the window following the onset of the light stimulus. The magnitude of the effect of strontium strongly correlated with baseline TC EPSC amplitude, suggesting that at LGN–FS synapses the largest the TC EPSC, the higher the number of release sites activated by a stimulus. Combined with lack of changes in CV following strontium chloride, these results suggest that LGN–FS synapses contain clusters of release sites that account for one third to one half of the amplitude of a TC EPSC (Bagnall et al., 2011). This interpretation fits well with the observation that the input–output curve of TC EPSCs in FS neurons has a steeper slope than that for Pyr neurons.

Our results demonstrate that LGN afferents engage target-specific presynaptic and postsynaptic mechanisms to activate feedforward excitatory and inhibitory circuits in L4 of the V1. LGN inputs in principle carry the same information. Target-specific synaptic properties may contribute at least in part to explaining why Pyr and FS neurons present distinct responsiveness to incoming stimuli (Bruno and Simons, 2002; Contreras and Palmer, 2003; Swadlow, 2002, 2003; Alonso and Swadlow,

Figure 6. Cell type-specific single-channel conductance and the number of open channels at LGN synapses in Pyr and FS neurons. **A**, Raw traces of TC EPSCs recorded from one Pyr neuron (black) overlaid by their average TC EPSC (red). The left panel shows an example of variation around the mean amplitude, while the right panel shows the peak-scaled variation around the mean (right). Vertical lines in the right panel indicate the region of the decay phase where the variance was measured. Light intensity, 0.1 mW/mm². **B**, Raw traces of TC EPSCs recorded from one FS neuron (black) overlaid by their average TC EPSC (red). The left panel shows an example of variation around the mean amplitude; while the right panel shows the peak-scaled variation around the mean (right). Vertical lines in the right panel indicate the region of the decay phase where the variance was measured. Light intensity, 0.1 mW/mm². **C** and **D** show the same analysis for two different neuron types. **C** is for pyramidal neurons and **D** is for FS neurons. Peak-scaled decay phase variance (bin size, 2 ms) plotted against the mean Pyr (left, black dots) and FS neurons (right, gray dots), and fitted with a parabolic curve (red). The curve equation is shown above the graphs. The fit was used to estimate values for i_u and N_o . **E**, Bar graph comparing i_u and N_o for Pyr (black) and FS (gray) neurons. Data are presented as the mean $\pm$ SE. Asterisks indicate significant differences.

2005; Cardin et al., 2007). FS neurons have lower input resistance and higher spike thresholds than Pyr neurons, but the large amplitude of the TC EPSCs they receive is quite effective at driving them above threshold. This is likely due to the organization of LGN inputs on FS neurons, which, according to our analysis, is consistent with the presence of clusters of release sites (Bagnall et al., 2011) with high release probability that activate few postsynaptic channels with large conductance. It is also consistent with the finding that FS neurons receive powerful direct inputs from retinotopically aligned LGN neurons (Zhuang et al., 2013). The powerful short-term depression, fast rise, and decay kinetics, and lack of NMDARs at LGN–FS synapses are likely contributing to evoking fast and precisely timed responses to visual stimuli. These properties likely narrow the temporal window of activation of these neurons (Jonas et al., 2004; Cardin et al., 2010). In our experiments, FS neurons were driven above threshold even by relatively weak light pulses. The large evoked TC EPSCs are consistent with previous reports that inhibitory neurons directly driven by the LGN respond more strongly than excitatory neurons to visual stimuli (Zhuang et al., 2013). Differently, LGN–Pyr neuron synapses rely on few powerful release sites that activate a large number of channels with small conductance. The effects of moderate short-term depression of these inputs, the presence of NMDARs on postsynaptic terminals, and the control of feedforward inhibition on their ability to fire action potentials may explain the favoring of Pyr neurons narrower tuning to visual stimuli. The target-specific properties of TC inputs in FS and Pyr neurons may provide a synaptic mechanism to explain the

Figure 7. Physiological evidence for target-specific presynaptic properties at LGN–V1 inputs. **A**, Left, Average TC EPSC recorded from one Pyr neuron before (baseline; black) application of SrCl_2 . Right, Single evoked TC EPSC recorded from the same Pyr neuron in the presence of 10 mM $\text{SrCl}_2/0$ mM CaCl_2 (gray). Light stimulus, 0.1 mW/mm^2 . **B**, Left, Average TC EPSC (black) recorded from one FS neuron before the perfusion of SrCl_2 . Right, Single evoked TC EPSC recorded from the same FS neuron in the presence of 10 mM $\text{SrCl}_2/0$ mM CaCl_2 (gray). Light stimulus, 0.1 mW/mm^2 . **C**, Effect of strontium on the amplitude of TC EPSCs for Pyr (left; dark gray) and FS (right; light gray) neurons; the average effect for both neuron types is shown in black in both plots. The dark gray and light gray markers indicate single recordings for Pyr and FS neurons, respectively. **D**, Distribution of the number of events in the 200 ms time window following the onset of the stimulus (the first evoked TC EPSC is excluded from the count). Black, Pyr; gray, FS; bin size, one event. **E**, CV of the amplitude of the first evoked TC EPSC before (filled bars) and after (open bars) application of $\text{SrCl}_2/0$ mM CaCl_2 for Pyr (black) and FS (gray) neurons. Data are presented as the mean $\pm$ SE. Asterisks indicate significant difference. **F**, Graphic representation of quantal analysis of TC EPSC amplitudes. Black open circles, Data from each Pyr neuron; black filled circle, Pyr average; gray open squares, data from each FS neuron; filled gray square, FS average; CV pre, baseline CV; CV post, CV after $\text{SrCl}_2/0$ mM CaCl_2 ; Mpre, mean baseline TC EPSC amplitude; Mpost, mean TC EPSC after $\text{SrCl}_2/0$ mM CaCl_2 ; n, number of release sites; p, release probability; q, quantal size; solid line, unity line. Average data are presented as the mean $\pm$ SE. **G**, Spearman rank order correlation of baseline TC EPSC amplitude in a Pyr neuron versus the change in TC EPSC amplitude in $\text{SrCl}_2/0$ mM CaCl_2 . **H**, Spearman rank order correlation of the baseline TC EPSC amplitude in FS neurons versus the change in TC EPSC amplitude in $\text{SrCl}_2/0$ mM CaCl_2 . For **G** and **H**: Rs, Spearman coefficients; p values < 0.05 indicate significant correlations.

broader tuning of FS neurons for stimulus orientation, direction, and spatial and temporal frequency of visual stimuli observed *in vivo* (Zhuang et al., 2013).

Coactivation of feedforward excitatory and inhibitory neurons by TC afferents is thought to be critical for regulating the precision of cortical neuron activation and cortical excitability. The properties of LGN–FS neuron inputs suggest that this feedforward inhibitory circuit provide a “window of excitability” (Swadlow, 2002, 2003; Wilent and Contreras, 2004) that ensures temporal precision of the transformation of synaptic inputs into neuronal firing rates (Wehr and Zador, 2003; Gabernet et al., 2005; Priebe and Ferster, 2005). The high spike reliability ratio of FS neurons in response to LGN terminal field activation supports the interpretation that LGN inputs successfully drive pyramidal neurons above threshold in a narrow window preceding the arrival of the inhibitory signal from FS neurons.

LGN–FS neuron synapses may also regulate circuit excitability by preventing runaway excitation (Swadlow, 2003; Sun et al., 2006). Indeed, the blockade of inhibition increased the ability of L4 Pyr neurons to fire action potentials. Finally, the activation of synapses containing NMDARs versus Ca^{2+} -permeable AMPARs provides a differential mode of regulation of intracellular calcium dynamics (Geiger et al., 1995; Dingledine et al., 1999), which can drive distinct signaling mechanisms in synaptic plasticity (Isaac et al., 2007; Gainey et al., 2009). As a consequence, the different mechanisms of activation of LGN inputs in Pyr and FS neurons may provide a substrate for connection-specific plasticity in L4 of the V1.

When our results are compared with findings from other sensory areas, it becomes evident that LGN inputs in L4 of the V1 present some similarities, but also significant differences. In S1 and A1, TC inputs in excitatory neurons activate AMPARs and NMDARs in postsynaptic terminals (Cruikshank et al., 2002; Rose and Metherate, 2005; Hull et al., 2009; Schiff and Reyes, 2012). The AMPAR-mediated components in excitatory neurons have properties similar to those we reported in V1. In S1 and V1, the NMDAR-mediated component of the TC EPSC can be detected at negative potentials, and these TC inputs activate NMDARs with low sensitivity to block Mg^{2+} (Fleidervish et al., 1998; Binshtok et al., 2006; Hull et al., 2009).

Similar to S1 and A1, the stimulation of LGN inputs in FS neurons evokes TC

EPSCs that have larger amplitudes than those in excitatory neurons (Hull et al., 2009; Cruikshank et al., 2007, 2010; Schiff and Reyes, 2012). TC inputs in FS neurons are mediated by GluRA2-lacking AMPA receptors in both S1 and V1. No NMDAR-mediated current contributed to TC EPSCs in FS neurons in V1; whereas in S1 and A1 TC EPSCs in FS neurons contain NMDAR-mediated currents, albeit smaller than those recorded from excitatory neurons (Hull et al., 2009). The data from V1 are consistent with observations in the prefrontal cortex, where TC EPSCs in 78% of FS neurons were mediated by GluR2-lacking AMPARs and lacked NMDAR-mediated currents (Wang and Gao, 2010). Differences in short-term depression were observed at TC inputs in excitatory neurons and FS neurons in S1 (Beierlein et al., 2003; but see Hull et al., 2009; Cruikshank et al., 2010; Viaene et al., 2011), A1 (Lee and Sherman, 2008; Schiff and Reyes, 2012), and V1 (this study), suggesting that distinct short-term dynamics are a general property of TC circuits, although the mechanisms engaged may differ across areas (Hull et al., 2009).

The organization of TC inputs also shows similarities and significant differences across sensory cortices. Our results suggest that LGN inputs in L4 of the V1 are mediated by a few powerful release sites, or clusters of release sites in the case of FS neurons, which is consistent with anatomical studies (Freund et al., 1985; Stratford et al., 1996; Ahmed et al., 1997; Erisir and Dreusicke, 2005). TC inputs are outnumbered by intracortical inputs (Peters and Payne, 1993; Ahmed et al., 1994, 1997; da Costa and Martin, 2009); however, feedforward stimuli are effectively propagated in cortical circuits. It was proposed that the amplification of signals by intracortical synapses may be necessary to propagate incoming activity in V1 and A1 (da Costa and Martin, 2011; Schiff and Reyes, 2012; Lee, 2013; Li et al., 2013). This presents significant differences with models of S1 activation. In S1, TC inputs in excitatory neurons have a quantal size similar to those of intracortical inputs (Gil et al., 1999; Schoonover et al., 2014). S1 activation by TC afferents is thought to depend on synchronous activation of numerous weak synapses (Bruno and Sakmann, 2006) with three times the number of release sites of intracortical synapses and a higher release probability (Hull et al., 2009; Schoonover et al., 2014). The similarities reported across sensory cortices likely underlie a set of general principles of TC circuit organization. The differences suggest that region-specific properties of TC inputs may depend on the specific demands of distinct sensory modalities.

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