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Fast-spike interneurons in visual cortical layer 5: Heterogeneous response properties are related to thalamocortical connectivity

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Abstract (250 words maximum, including citations, 250 words now)

Layer 4 of rabbit V1 contains fast-spiking GABAergic interneurons (suspected inhibitory interneurons, SINs) that receive potent synaptic input from the LGN and generate fast, local feed-forward inhibition. These cells display receptive fields with overlapping ON/OFF subregions, non-linear spatial summation, very broad orientation/directional tuning, and high spontaneous and visually-driven firing rates. Such fast-spike interneurons are also found in layer 5 (L5), which receives a much sparser input from the LGN, but the response properties and thalamocortical connectivity of L5 SINs are relatively unstudied. Here, we study L5 SINs in awake rabbits (both sexes) and compare their response properties with previously studied SINs of L4. We also assess thalamocortical connectivity of L5 SINs, examining both cross-correlation of retinotopically aligned LGN-SIN spike-trains and L5 SIN responses to electrical stimulation of the LGN. These analyses confirmed that many L5 SINs, like L4 SINs, receive a strong and fast monosynaptic drive from the LGN. Moreover, these LGN-connected L5 SINs had response properties that were similar to those of L4 SINs and were predominantly found in the upper half of L5. By contrast, L5 SINs with longer synaptic latencies to LGN stimulation displayed (1) sharper orientation tuning, (2) longer visual response latencies, (3) lower spontaneous and (4) visually-driven firing rates, and (5) were found in the deeper half of L5. We suggest that the long-latency synaptic responses in such L5 SINs reflects a multi-synaptic intracortical pathway that generates a different constellation of response properties than seen in L5 SINs that are driven directly by LGN input.

Significance Statement (96 words):

Fast-spike GABAergic interneurons are found across the entire depth of the visual cortex but may have very different response properties and functions in each cortical

layer depending on a host of factors that are specific to the different layers. In layer 4, they are known to receive potent synaptic input from the thalamus and generate fast, local feed-forward inhibition. In layer 5, they are thought to receive less direct thalamic input and be strongly dominated by intracortical input. Here, we show that some layer-5 interneurons receive powerful monosynaptic input from LGN and have visual response properties more similar to layer 4 interneurons than those receiving little direct LGN input.

Introduction (650 words limit, 531 words)

Fast spike GABAergic interneurons are found in layers 2-6 of sensory neocortex. However, studies of their sensory response properties and thalamocortical connectivity have been largely limited to layer 4 (L4) (Swadlow and Gusev, 2001; Gabernet et al., 2005; Stoelzel et al., 2008; Zhuang et al., 2013; Bereshpolova et al., 2020; Liew et al., 2021). In L4 of rabbit visual cortex (V1), putative fast spike GABAergic interneurons (suspected inhibitory interneurons, SINs) display “complex” receptive fields (RFs), with highly overlapped ON and OFF sub-regions, very broad (or no) orientation tuning, and nonlinear spatial summation to drifting visual stimuli (F1/F0 ratios of < 1). In addition, L4 SINs in rabbit V1 receive a highly convergent input from the retinotopically aligned region of the lateral geniculate nucleus (LGN). Similarly, SINs of both rodent and rabbit somatosensory barrel cortex (S1) receive strong convergent inputs from somatotopically aligned regions of ventrobasal thalamus (Swadlow, 2003; Bruno and Simons, 2002). These strong thalamocortical inputs to sensory cortical L4 SINs are thought to mediate a rapid and potent feedforward inhibition onto local spiny cells that shapes both temporal and spatial response properties (Ferster and Miller, 2000; Sun et al., 2006; Cruikshank et al., 2007).

L5 of sensory neocortex also contains a significant population of fast-spike GABAergic interneurons, but the density of thalamocortical inputs to L5 is relatively low when compared to L4, both in V1 and S1 (Stoelzel et al., 2008; El-Boustani et al., 2020; Ji et al., 2016; Zhuang et al., 2021). However, L5 does receive a rich input from superficial cortical layers and, according to the “canonical” circuitry of Gilbert and Wiesel (1983), the inputs from superficial layers dominate the visual responses of L5 neurons (Petreanu et al., 2007; Zarrinpar and Callaway, 2016; Quiquempoix et al., 2018; but see Constantinople and Bruno, 2013). Given such differences between L4 and L5 in thalamocortical and intracortical circuitry, here we ask how the visual response properties and thalamocortical connectivity of SINs in L5 and L4 differ. To do this, we study the visual response properties of L5 SINs in rabbit V1 and compare them with the properties of L4 SINs that we have previously studied using near identical methods (Zhuang et al., 2013). We also assess the synaptic connectivity of L5 SINs with the LGN using both cross-correlation analysis of retinotopically aligned LGN-SIN pairs, and electrical stimulation of the LGN. We found that response properties of most L5 SINs are remarkably similar to the properties of previously studied L4 SINs. However, there was considerably more heterogeneity in the response properties of the L5 SINs, some

of which were related to differences among L5 SINs in thalamocortical connectivity. For example, L5 SINs with longer synaptic latencies to thalamic stimulation were found deeper in L5 and were more sharply tuned to stimulus orientation than those with short synaptic latencies. Moreover, cross-correlation analysis showed that only L5 SINs with short synaptic latencies to thalamic stimulation were monosynaptically connected to retinotopically aligned LGN neurons. Thus, our results indicate fundamental similarities in the response properties of L4 and L5 fast-spike GABAergic interneurons, but also differences that are related to thalamocortical connectivity and depth within L5.

Materials and Methods

We recorded neurons in LGN and retinotopically aligned regions of the primary visual cortex (V1) from two male and one female awake adult Dutch-Belted rabbits. All experimental procedures were conducted in accordance with National Institutes of Health guidelines and were approved by the University of Connecticut Animal Care and Use Committee.

Animal preparation and experimental design

The general surgical procedures for chronic recordings are similar to those previously published by our group (Bereshpolova et al., 2006; 2020; Zhuang et al., 2013; Su et al., 2024) and are reported briefly here. Skin and fascia above the skull were removed under Ketamine/Xylazine/Acepromazine (40/5/2 mg/kg) anesthesia using aseptic procedures. A head-restraint stainless-steel bar was affixed to the skull with acrylic cement. The exposed portions of skull were covered with medical grade silastic compound to provide a buffer for the wound margins from the acrylic cement. Subsequently, microelectrodes implantation was performed after two weeks of recovery. A seven-channel concentric, independently moveable microelectrode system (Swadlow et al., 2005a) was chronically implanted in the monocular region of V1 (Fig. 1A). Single unit activities and cortical local field potentials (LFP) were obtained using 40 μm diameter, quartz-insulated, platinum-tungsten electrodes tapered and sharpened to a fine tip with impedance 1.5 – 3 Mohm. Multi-unit activity from superficial layers of the superior colliculus (SC) was simultaneously recorded by a similar 3-channel microdrive system. Three stimulation electrodes (parylene-c insulated platinum/iridium micro wire) were implanted within the retinotopically aligned region of the LGN for identification of fast-spike interneurons in V1 and three stimulation electrodes were implanted in the SC for antidromic identification of corticotectal neurons. The experiment also involved recording hippocampal EEG through three electrodes implanted above and below the CA1 layer to monitor brain state. Cortical EEG was recorded using one electrode placed above the somatosensory cortex for additional brain state monitoring. All electrophysiological activity was acquired using a Plexon data acquisition system.

To investigate thalamocortical connectivity between LGN neurons and L5 SINs, a 7-channel microelectrode system (Swadlow et al., 2005a), akin to the one detailed earlier, was implanted in the LGN for chronic recordings. A small diameter craniotomy (~ 1mm) was performed over retinotopically aligned region of V1. A fine-diameter single electrode was moved through the cortical depth by a Narishige manipulator to record L5 SINs. The spontaneous activities of both LGN neurons and the retinotopically aligned L5 SINs were simultaneously recorded for subsequent cross-correlation analysis. For each pair of LGN and L5 SIN, a minimum of 5000 spontaneous LGN spikes were collected to assess thalamocortical connectivity, providing valuable insights into the functional interactions between these neural populations.

Identification of layer 5

The identification of cortical layers relied on detecting the depth of the reversal of stimulus-evoked LFPs in V1. These LFPs were induced by a brief (20 ms) flash of a 15–20 degrees circular bright visual stimulus. Histological evidence (Stoelzel et al., 2008) showed that the upper border of layer 5 was ~ 500 µm below the reversal point, and the upper border of layer 6 was ~ 300 µm below the upper border of layer 5 (Stoelzel et al., 2017). Therefore, layer 5 was estimated from 500 to 800 µm below the reversal point of the flash-evoked potential. In addition, we identified corticotectal (CTect) neurons by antidromic stimulation. Because the vast majority of CTect neurons in rabbits (as in other mammals) are found in L5, if a second neuron is found very near to a CTect neuron, this provides confirmatory evidence that the second neuron is also in layer 5.

Identification of SINs

SINs were identified by a high-frequency discharge of 3 or more spikes to the thalamic stimulus, with a minimal inter-spike interval of less than 1.667 ms (600 Hz, Fig. 1B, Swadlow, 1989, 2003; Zhuang et al., 2013). For each cell, we electrically stimulated the thalamus (rectangular voltage pulses, 0.2 ms duration, range: < 1 V – 45 V) and measured the stimulus threshold, latency of the first spike, and minimum interspike interval. Only SINs estimated to be in L5 (recorded within 500 to 800 µm below the reversal point of the flash-evoked potential) were included in this study. SINs also have short spike waveform duration, but this was not used as an identification criterion (See Results & Fig. 1C).

Visual stimulations and eye movement control

Visual stimulation protocol was similar to those previously published (Zhuang et al., 2013; Su et al., 2024). Visual stimuli were generated through a custom-made program (Visual C++, DirectX 7) and displayed on a CRT monitor (NecMultiSync, primary monitor, 40 x 30 cm, mean luminance: 48 cd/m2, refresh rate: 160Hz). The reverse correlation method (Jones and Palmer, 1987) was employed to generate maps of RFs from sparse noise stimuli, consisting of light and dark squares. During testing of visual response properties, the RF center was continuously monitored by dynamic calculation of RF position from multi-unit recordings in the SC. Sparse noise stimulation

was presented on a second LCD monitor, positioned next to the main monitor. If an eye movement occurred during testing, the relation between the RF center of the cortical cell and the SC multiunit RF center was used to dynamically place the stimulus on the cortical RF (See Su et al., 2024). A high-frequency (220 Hz) infrared camera system (ViewPointEyeTracker® system, Arrington Research, Inc.) was also used to track pupil size and position during the recording session.

Neurons' optimal orientation/direction, spatial frequency, temporal frequency, size, and contrast were measured using circular drifting sinusoidal gratings. Following the assessment of a neuron's response to optimal drifting grating, one of the four tuning properties (orientation, spatial frequency, temporal frequency, and contrast) was pseudorandomly tested while keeping the other parameters at their optimal values. Each grating parameter was presented for 0.5 s to 2 s, with a 1 s gap in between.

Responses were classified in sustained or transient using an optimal flashing stationary stimulus, which were presented on the center on RF for 2 s, with a 2 s gap during the alert brain state.

Data analysis

The offline data analysis methods have been reported before (Zhuang et al., 2013; Su et al., 2024) and are described briefly here. Spikes from single neurons were isolated during the recording session and verified offline with Plexon cluster analysis software. All data analysis was performed with NeuroExplorer (NexTechnologies, Inc.), MATLAB (The MathWorks, Inc.), and Python (Python Software Foundation).

The spatial structure of L5 SINs' RFs was assessed using two indices: the Local Similarity Index (LSI, DeAngelis et al., 1999; Usrey et al., 1999; Alonso et al., 2001) and Sign Index (Jin et al., 2008; Van Hooser et al., 2013). LSI was calculated as $(RF_{ON} \cdot RF_{OFF})/\sqrt{(RF_{ON} \cdot RF_{ON})(RF_{OFF} \cdot RF_{OFF})}$. It quantifies the degree of spatial segregation between ON (RF_{ON}) and OFF (RF_{OFF}) subfields, with a value of 0 indicating complete separation and 1 indicating perfect superimposition. The Sign Index was calculated as $(\sum R_{ON(i,j)} - \sum R_{OFF(i,j)})/(\sum R_{ON(i,j)} + \sum R_{OFF(i,j)})$. $RF_{ON(i,j)}$ is the maximum response of the ON subfield while the $RF_{OFF(i,j)}$ represents the maximum response of the OFF subfield. Sing index evaluates the dominance of ON or OFF subfields, ranging from -1 for OFF-dominated to 1 for ON-dominated RFs, with values close to zero representing a balance between ON and OFF polarity. SINs with a Sign Index absolute value from 0 to 0.3 were classified as having balanced ON-OFF subfield dominance. Cells with a Sign Index absolute value from 0.3 to 0.7 were considered as having a stronger ON or OFF subfield. Those with a Sign Index absolute value from 0.7 to 1 were classified as having a single ON or OFF subfield. The subfield width was measured along the orientation perpendicular to the cell's optimal orientation from the dominant subfield (Zhuang et al., 2013; Su et al., 2024). To ensure the quality of the RFs, each single SIN was mapped at least twice to confirm repeatability without any eye movement. In most cases, SINs were mapped at least five times during the recording session to verify RF repeatability. We selected recording files based on neuron isolation quality and the number of spikes

collected for further analysis. SINs that did not produce repeatable RFs due to eye movement, noise, or insufficient spike count were excluded from the data set.

The response latency was calculated from the neurons' peristimulus time histograms (PSTHs) to optimal flash stimuli. The latency is defined as the time at which the PSTH was smoothed by a boxcar filter with a 30 ms sliding window, and it was determined as the point when it first exceeded 40% of its maximum value (Jin et al., 2011). The sustained/transiency of the neuronal response was tested using a flashed stimulus optimized to the SINs' preferred polarity and size. The sustained index was calculated by the ratio of the neuron's maintained response to the baseline activity when the animal is alert. PSTHs were generated with a bin size of 5 ms to measure the maintained response, which was defined as the mean firing rate within 0.5 s – 1.0 s after stimulus onset, while baseline activity was measured as the mean firing rate from -0.5 s to 0 s before stimulus onset. The alert brain state was indicated by hippocampal "theta" activity (5 – 7 Hz) and desynchronized cortical EEG (Bezdudnaya et al., 2006; Zhuang et al., 2013).

The mean firing rate (F0), first harmonic component (F1) of the PSTHs, F1/F0 ratios, and Fano Factors were measured from the SINs' response to the optimal drifting grating stimuli. The analysis of all tuning properties followed the same methodology as described earlier (Su et al., 2024). Orientation tunings were fitted by von Mises distribution functions responses (Nowak et al., 2008; Zhuang et al., 2013). The orientation selectivity index (OSI), and direction selectivity index (DSI) were calculated as $(R_{pref} - R_{orth})/(R_{pref} + R_{orth})$, and $(R_{pref} - R_{null})/(R_{pref} + R_{null})$. Here, Rpref denotes the neuron's response in the preferred orientation/direction, Rorth signifies the neuron's average responses in the stimulus directions 90° away from the preferred direction, and Rnull represents the neuron's response to the opposite of the preferred direction. Both measurements (OSI and DSI) have values ranging from 0 to 1, with higher values meaning stronger orientation selectivity and direction selectivity, respectively. Contrast tunings were tested on 8 different contrasts ranging from 1% to 95% (Cano et al., 2006; Zhuang et al., 2013). The F0 responses were fitted by a hyperbolic model (Albrecht and Hamilton, 1982; Cano et al., 2006; Zhuang et al., 2013), without high contrast suppression $y = R_0 + A * x^n / (C^n + x^n)$, or with high contrast suppression: $y = R_0 + A * x^n / (C^{sn} + x^{sn})$. Here, R0 is the baseline activity of the neuron, A is the response amplitude, x is the contrast that we tested, and C is the contrast generating the half-maximum response (C50). Only the fitting curves with goodness of fit value higher than 0.5 were included for further analysis.

The connectivity within simultaneously recorded pairs of LGN-L5 SINs was investigated using cross-correlation analysis (Liew et al., 2021; Reid and Alonso, 1995; Swadlow and Gusev, 2001; Bereshpolova et al., 2020). The crosscorrelogram depicted the correlation between the count of L5 SINs spikes occurring within the ± 10 ms time window (with 0.2 ms bins) and the LGN spikes. A significant peak is defined as at least two out of three successive bins in the peak exceeded the 0.01 confidence level at intervals of 1-4 ms (Fig. 1D). To evaluate the functional connectivity between the LGN

cell and the L5 SINs neurons, an efficacy value is calculated. Efficacy and contribution values (Levick et al., 1972; Swadlow, 1995; Bereshpolova et al., 2020) are calculated by counting the number of spikes that occurred in the L5 SINs during the short time window of ± 0.6 ms from the peak (baseline subtracted) divided by the number of the triggering LGN spikes (efficacy) or by the number of the L5 SINs spikes (contribution).

Statistical analysis

Statistical analyses were performed using MATLAB (MathWorks). The results section provided details on the type of statistical test utilized. Data are provided as Mean ± SEM.

Results:

Identifying characteristics of L5 SINs

L5 SINs, like those of L4, are identified by their high frequency discharge (> 600 Hz) to electrical stimulation of the thalamus (Zhuang et al., 2013; Bereshpolova et al., 2020; Fig. 1B). Although not a defining criterion, all SINs had spikes of short duration. Figure 1C shows the distribution of the spike waveform durations (measured from trough to peak) of the L5 SINs, L4 SINs (from Zhuang et al., 2013) and corticotectal (CTect) neurons (from Su et al., 2024). CTect neurons were identified by their antidromic activation following electrical stimulation of the SC (Bishop et al., 1962; Swadlow and Weyand, 1987). The spike waveform durations of L5 SINs are very similar to the spike waveform durations of L4 SINs (L5 SINs vs. L4 SINs: 0.21 ± 0.01 ms vs 0.19 ± 0.01 ms, Wilcoxon rank sum test, $p = 0.107$), but the waveform durations of L5 SINs are ~ ½ those of the CTect neurons (0.21 ± 0.01 ms vs. 0.38 ± 0.01 ms, Wilcoxon rank sum test, $p = 4.803e^{-24}$, Fig. 1C).

Spatial receptive field properties of L5 SIN:

We tested the spatial RFs of 65 L5 SINs to small stationary flashing light and dark spots using methods of reverse correlation (Methods). The RFs of fifty-six of these cells (86%) consisted of highly overlapping ON and OFF zones (Fig. 2A1). The RFs of six L5 SINs consisted of a single ON (four cells, Fig. 2A2) or OFF (two cells, Fig. 2A3) zone. Two L5 SINs responded to flashing spots only with suppression of spontaneous firing, and one remaining cell was not driven by visual stimulation (Table 1). Two SINs with suppressive RFs and one non-responsive SIN were not tested with drifting grating stimulations for their visual properties, and thus are not included in most of the subsequent analyses. Notably, the most common type of RF described in Fig. 2A1 (with highly overlapping ON/OFF subfields) is the only type of spatial RF previously seen in L4 SINs (Zhuang et al., 2013; Bereshpolova et al., 2020). The measure “Sign Index” (Jin et al., 2011; Van Hooser et al., 2013; Methods) was used to quantify the strength of ON and OFF subfields. Fig. 2B shows the distribution of the Sign Index values for L5 SINs. The majority of L5 SINs have Sign Index values around -0.3 to 0.3, indicating they

have roughly balanced ON and OFF responses (see Methods). The Sign Index values of -1 and +1 indicate pure OFF and ON responses, respectively. Because the L5 SINs are spontaneously active, there is often considerable noise in the RF plots. For this reason, RFs were classified as having a single ON or OFF subfield if Sign Index values were > 0.7 or < -0.7, respectively. To quantify the degree of overlap between the ON and OFF subfields, the measure "Local Similarity Index" (LSI, DeAngelis et al., 1999; Usrey et al., 1999; Alonso et al., 2001, Methods) was calculated. Fig. 2C shows the distribution of LSI for L5 SINs with both an ON and an OFF subfield. All of these L5 SINs have LSI > 0.3 (mean = 0.59 ± 0.02), indicating their ON and OFF subfields are overlapping to some extent.

Response properties of L5 SINs: Comparisons with L4 SINs

In addition to their spatial RF structure, L5 SINs share many similarities with the L4 SINs. Importantly, they both show nonlinear spatial summation in the responses to optimal drifting sinusoidal gratings (F1/F0 ratio < 1). The linearity of spatial summation was measured as the ratio between the modulated (F1) to the unmodulated (F0) responses. A response is considered linear when the F1/F0 ratio > 1, and nonlinear when the ratio < 1 (Skottun et al., 1991; Movshon et al., 1978b, a; Martinez and Alonso, 2003; Zhuang et al., 2013). All but two L5 SINs responded to their optimal drifting grating stimulation in a nonlinear manner. Fig. 3A is an example of an L5 SIN responding to near-optimal drifting grating, with F1/F0 ratio of 0.07. The evoked F0 response increases during the stimulus presentation, but it was weakly modulated by the sinusoidal drifting grating stimulation. By contrast, classic "simple" cells of L4 respond in a highly modulated linear manner to an optimal drifting visual grating. Fig. 3B is an example of such a simple cell (in layer 4 of rabbit V1) responding to its optimal visual stimulation, with F1/F0 ratio of 1.50. Fig. 3C shows the distribution of the F1/F0 ratio of L5 SINs, all but two of which have an F1/F0 ratio of <1.0 (Fig. 3C, upper panel, mean = 0.28 ± 0.03). We compare the L5 SINs to the F1/F0 ratio of L4 simple cells (Fig. 3C, lower panel, mean = 1.53 ± 0.03) that we previously studied (Zhuang et al., 2013). Visual cortical neurons with F1/F0 values of < 1.0 are frequently classified as "complex" cells and those with values > 1.0 as "simple" cells (Movshon et al., 1978b, a; Martinez and Alonso, 2003; Briggs and Usrey, 2009; Hawken et al., 2020). Therefore, like the L4 SINs, all but two of the L5 SINs would be considered as complex cells based on this classification. Notably, the spatial RF of the two L5 SINs with F1/F0 ratios of > 1 had only a single subfield.

Figure 4 shows other similarities (A-E, all significant at p > 0.05) as well as differences (F-L) between the L5 SINs and previously studied L4 SINs (from Zhuang et al., 2013). Thus, the degree of spatial overlap in the ON and OFF subfields of the L5 and L4 SINs was similar (LSI: L5 vs L4 = 0.59 ± 0.02 vs. 0.66 ± 0.02, Wilcoxon rank sum test, p = 0.067, Fig. 4A, high LSI values mean greater overlap). Both populations also have similarly high evoked unmodulated (F0) responses (L5 vs L4 = 50.52 ± 3.91 spks/s vs. 59.76 ± 4.20 spks/s, Wilcoxon rank sum test, p = 0.061, Fig. 4B) to a drifting

visual grating, and both display similar response reliability to optimal drifting grating stimulation (Fano Factor: L5 vs L4 = 1.71 ± 0.18 vs. 1.33 ± 0.08, Wilcoxon rank sum test, p = 0.185, Fig. 4C, high Fano Factor values mean less reliable response). Both L4 and L5 SINs are also poorly tuned to stimulus direction (DSI: L5 vs L4 = 0.10 ± 0.02 vs. 0.15 ± 0.02, Wilcoxon rank sum test, p = 0.126, Fig. 4D, high DSI values indicate greater directional specificity), and both populations include cells that generate sustained responses to stationary flash stimuli (sustained index: L5 vs L4 = 2.16 ± 0.42 vs 1.48 ± 0.19, Wilcoxon rank sum test, p = 0.573, Fig. 4E, higher sustained index higher values indicate greater sustained response).

By contrast, Fig. 4 F-L shows that some L5 SINs show significant differences in some of their response properties compared to the L4 SINs. More L5 SINs than L4 SINs are selective for stimulus orientation (OSI: L5 SINs vs. L4 SINs: 0.25 ± 0.02 vs. 0.16 ± 0.01, Wilcoxon rank sum test, p = 0.006, Fig. 4F, higher OSI values indicate greater selectivity for stimulus orientation). Both L5 and L4 SINs have relatively high spontaneous activity, but the L5 SINs have significantly lower spontaneous activities than L4 SINs (L5 SINs vs. L4 SINs: 14.46 ± 0.98 spks/s vs. 21.96 ± 2.18 spks/s, unpaired t-test, p = $7.718e^{-04}$, Fig. 4G). The modulated responses (F1) are less than ½ the strength in L5 than in L4 SINs, (L5 SINs vs. L4 SINs: 14.10 ± 1.86 spks/s vs. 32.97 ± 3.73 spks/s, Wilcoxon rank sum test, p = $1.358e^{-06}$, Fig. 4H). Although the great majority of the L4 and L5 SINs respond to drifting sinusoidal gratings in a nonlinear manner (F1/F0 ratio < 1, Fig. 3), the L5 SINs have much lower F1 responses and, therefore, they also have lower F1/F0 ratios than the L4 SINs (L5 SINs vs. L4 SINs: 0.28 ± 0.03 vs. 0.64 ± 0.04, Wilcoxon rank sum test, p = $1.813e^{-07}$, Fig. 4I). The L4 SINs respond at short latencies to visual flash stimuli, with a median ~28.16 ms. However, some L5 SINs respond at long latencies (L5 SINs vs. L4 SINs: 37.59 ± 3.65 ms vs. 28.65 ± 0.93 ms, Wilcoxon rank sum test, p = 0.017, Fig. 4J). Contrast sensitivity measured as the contrast that elicited half of the maximum response (C50, lower values indicate greater contrast sensitivity) is also lower in L5 than L4 SINs (L5 SINs vs. L4 SINs: 17.63 ± 2.70 % vs. 10.83 ± 1.94 %, Wilcoxon rank sum test, p = 0.011, Fig. 4K). The RF sizes of the L5 SINs and L4 SINs are also different. The dominant subfield widths are significantly smaller in L5 than L4 SINs (L5 SINs vs. L4 SINs: 6.62 ± 0.39 deg vs 8.35 ± 0.53 deg, Wilcoxon rank sum test, p = 0.011, Fig. 4L).

Figure 4F-L show differences between L5 and L4 SINs: F, Distribution of OSI for L5 SINs and L4 SINs. The majority of L5 and L4 SINs are broadly tuned for stimulus orientation, but some L5 SINs are orientation selective. G, The spontaneous activities are significantly lower in L5 than L4 SINs. H, Distribution of evoked F1 responses for L5 and L4 SINs. The L5 SINs have lower evoked F1 responses than the L4 SINs, indicating weaker modulation to optimal drifting sinusoidal gratings. I, Distribution of F1/F0 ratio for L5 SINs and L4 SINs. Although L5 SINs are less modulated to optimal drifting sinusoidal gratings than L4 SINs, their maximum evoked responses are similar (F0 response, Fig. 4B). The F1/F0 ratio is much lower in L5 than L4 SINs. J, Distribution of visual latency to flash stimuli for L5 and L4 SINs. L4 SINs respond at very short

latencies to flash stimuli, while some L5 SINs respond much more slowly than L4 SINs. K, Distribution of contrast sensitivity measured as C50 (%) for L5 SINs and L4 SINs. The L5 SINs have higher C50 values than the L4 SINs, indicating less sensitivity to stimulus contrast. L, Distribution of dominant RF width for L5 SINs and L4 SINs. The L5 SINs have smaller dominant RF widths than the L4 SINs.

L5 SIN properties related to their synaptic latencies to LGN stimulation.

All of the L5 SINs studied responded to electrical stimulation of the LGN with a burst of high-frequency spikes, but the latency of the first spike in this discharge differed considerably among cells. Figure 5A shows the distribution of the synaptic latencies of L5 SINs to electrical stimulation of the LGN (mean = 2.35 ms, range = 1.4 – 5.0 ms). As described in Methods, we have estimated the depth of L5 as extending from 500um – 800 µm below the reversal point of the flash-evoked LFP. Fig. 5B shows that the depths of the L5 SINs were loosely correlated with their synaptic latencies to thalamic stimulation. SINs located in deeper L5 tended to have long synaptic latencies ($r = 0.286$, $p = 0.021$). The synaptic latency of the L5 SIN was also related to visual response properties. L5 SINs with longer synaptic latencies were more selective for stimulus orientation ($r = 0.384$, $p = 0.012$, Fig. 5C). Fig. 5D shows visual response latency to a flash stimulus, with longer visual response latencies seen in cells with longer synaptic latencies to the electrical stimulus ($r = 0.493$, $p = 0.007$). L5 SINs with longer latencies to the electrical stimulus have lower spontaneous firing rates ($r = -0.421$, $p = 0.001$, Fig. 5D) and lower response rates to optimal drifting grating stimuli ($r = -0.465$, $p = 0.001$, Fig. 5E). Synaptic latency to electrical stimulation was not significantly related to their linearity of spatial summation (F1/F0 ratio), modulated F1 response, direction selectivity, contrast sensitivity, response reliability, or dominant RF width (figures not shown).

Thalamocortical synaptic inputs and their relationship to L5 SIN response properties

It is tempting to speculate that L5 SINs responding at short synaptic latencies to electrical stimulation of the LGN receive direct monosynaptic input and, conversely, those responding at long latencies receive only weak or indirect multisynaptic LGN input. It is well known, however, that evidence from electrical stimulation alone is not sufficient to justify such conclusions since (a) the electrical stimulus may activate afferents that do not originate at the stimulation site and (b) the electrical stimulus may activate corticofugal fibers, generating antidromic impulses that invade collaterals that synapse on and activate the cortical SINs.

SINs of L4 in both V1 and S1 have been shown to receive potent monosynaptic thalamic inputs (Swadlow, 1995, 2002; Zhuang et al., 2013). However, LGN afferents synapse much more extensively in L4 than in L5 and it is not known the extent to which L5 SINs receive significant monosynaptic LGN inputs. We were interested in this because the differences in visual properties (as shown in Figure 4) between L5 SINs

and L4 SINs could potentially be attributed to whether L5 SINs receive weaker/fewer direct inputs from the LGN.

Here, we used cross-correlation methods to investigate whether the L5 SINs receive monosynaptic inputs from the LGN, the efficacy of such inputs, and how such input might relate to their response to electrical stimulation of the LGN and to their visual response properties. Using this method in sensory thalamocortical systems, requires achieving a precise topographic (retinotopic) alignment between the thalamic and cortical neurons. Here we only investigated and further analyzed the LGN-L5 SIN pairs that are in retinotopic alignment, such that the LGN and SIN RF centers were separated by a distance equivalent to <80% of the diameter of the LGN RF center (see Methods). Based on this criterion for retinotopic alignment, we found 13 LGN-L5 SIN pairs that showed evidence of synaptic connectivity. Nineteen pairs that were retinotopically aligned to an equivalent degree were not connected. Figure 6A-M shows cross-correlograms of each of the LGN-L5 SIN pairs that met our criteria for monosynaptic connectivity (see Methods). Each cross-correlogram shows a brief and abrupt peak in SIN spike probability within 1- 4 ms after the LGN spikes (see Methods). Efficacy (E) and contribution (C) values were used to quantify the strength/impact of the connection and were labeled in each of the LGN-L5 SIN cross-correlogram. Note that although Fig. 6 shows 13 connected LGN-L5 SIN pairs, only 10 SINs are represented by this figure. This is because, in cases A and B, a single L5 SIN was studied with two LGN concentric cells, one ON-centered and the other OFF-centered. Similarly, in case C and D, and in case G and H, two other SINs both receive input from two different LGN neurons (again, one ON-center and the other OFF-center). Our data in Fig. 6 seems to indicate that LGN neurons with OFF-center RFs exhibited greater efficacy onto L5 SINs. However, these data include one OFF-center LGN neuron that made synaptic contact with four L5 SINs (connections depicted in Fig. 6A, C, E, and I are from the same LGN off-center neuron). Due to the over-representation of this single LGN neuron in our sample, our dataset remains too limited to conclude whether LGN neurons with off-center RFs provide stronger input to L5 SINs compared to LGN cells with on-center RFs.

All of the connected L5 SINs were recorded between 520 and 650 µm beneath the reversal point of the flash evoked potential (Fig. 6N, right column). Moreover, most were recorded very near to CTect neurons, which, in rabbits, are predominantly found in the superficial ½ of layer 5 (Su et al., 2024; Swadlow and Weyand, 1981). Thus, seven of the 10 connected L5 SINs were recorded either simultaneously with a CTect neuron, sequentially within 60 µm of a CTect neuron, or ~ 200 µm below a CTect neuron using the same electrode during a single penetration. This provided additional evidence, aside from the depth profile, that these SINs are located in L5. Although the other three connected L5 SINs were not recorded with a CTect in the same penetration, they were all located near the middle of L5 (Fig. 6N, open circles). Therefore, the existence of CTect neurons in close proximity to the monosynaptically connected SINs with LGN affirmed the location of these SINs in L5.

Next, we asked whether the response properties of the 10 LGN-connected SINs differed from the properties of the 19 SINs that, according to our cross-correlation analysis, were not connected despite being retinotopically aligned to an equal degree.

We observed the following differences between LGN-connected vs. non-connected L5 SINs that were aligned to an equal degree: (1) the cortical depth at which they were found, (2) the amplitude of the LGN spike-triggered LFP (triggered by spikes of the single LGN cell under study, Methods) in the vicinity of the SIN, (3) the spontaneous activity of the SINs, and (4) their synaptic latencies to LGN electrical stimulation. Thus, the L5 SINs that received monosynaptic LGN input were all located in the upper half of L5, while none of the L5 SINs located in the lower half of L5 received monosynaptic LGN input (depth: connected vs. not connected: 573 ± 17 µm vs. 693 ± 19 µm, unpaired t-test, $p = 1.369e^{-04}$, Fig. 7A). The amplitude of LGN spike-triggered LFP at the vicinity of the connected L5 SINs is ~ 6 times higher than the amplitude of the spike-triggered LFP near the non-connected L5 SINs (connected vs. not connected: 10.00 ± 2.37 µV vs. 1.47 ± 0.51 µV, Wilcoxon rank sum test, $p = 4.067e^{-04}$, Fig. 7B). Also, the LGN connected L5 SINs have higher spontaneous activity than those of the non-connected L5 SINs (connected vs. not connected: 18.01 ± 1.62 spks/s vs. 10.76 ± 1.34 spks/s, unpaired t-test, p = 0.003, Fig. 7C). All connected L5 SINs have short (≤ 2.4 ms) synaptic latency to thalamic electrical stimulation (indicative of monosynaptic activation, Bereshpolova et al., 2020), while the synaptic latency of the non-connected L5 SINs can be much longer (connected vs. not connected: 2.02 ± 0.08 ms vs. 2.76 ± 0.31 ms, unpaired t-test, p = 0.041, Fig. 7D).

Discussion (1500 words, 1500 words limit)

Fast-spike interneurons are found throughout sensory neocortex, comprising about ½ of the inhibitory neurons in many cortical areas (Rudy et al., 2011; Tamamaki et al., 2003). These parvalbumin-expressing interneurons are morphologically and biochemically distinct from neighboring excitatory cells, and from other classes of GABAergic interneurons (Markram et al., 2004; McBain and Fisahn, 2001; Tremblay et al., 2016). They are also physiologically distinct from excitatory neurons, displaying short-duration action potentials, high firing rates, and distinct RF properties (Simons, 1978; Swadlow, 1988, 1989, 1990, 1991; Hirsch et al., 2003; Nowak et al., 2008). They also respond potently to thalamocortical inputs and generate fast and powerful local feed-forward inhibition (Gibson et al., 1999; Cruikshank et al., 2007; Porter et al., 2001; Swadlow, 2003).

Cross-correlation studies in L4 barrel cortex of rats and awake rabbits (Bruno and Simons, 2002; Swadlow and Gusev, 2001) have shown that fast-spike neurons/SINs receive a highly convergent synaptic input that drives many neurons (~ 60%) in the aligned thalamic “barreloid”. Notably, although most neighboring spiny neurons in L4 are sharply tuned to the direction of whisker displacement, fast spike neurons/SINs are very broadly tuned, despite considerable directional selectivity in their thalamic afferents. This broad tuning is thought to result from strong convergence of

many thalamic neurons with a broad range of directional preferences onto the interneurons (Alonso and Swadlow, 2005; Swadlow, 2003; Swadlow et al., 2005b). In rabbit V1, a similar unselective convergence of LGN neurons onto L4 SINs occurs (Bereshpolova et al., 2020). Like SINs of L4 barrel cortex, SINs of L4 in V1 are very broadly tuned to some stimulus parameters (visual stimulus direction and orientation), and the observed high degree of thalamocortical convergence is thought to be related to these broad tuning characteristics (Zhuang et al., 2013).

L5 of V1 also has many fast-spike interneurons that potently inhibit their neighbors (Xiang et al., 2002). However, the axonal arborization and synaptic drive of LGN axons in L5 is considerably sparser than in L4 (Humphrey et al., 1985; Ji et al., 2016; Stoelzel et al., 2008; Zhuang et al., 2021). Moreover, L5 receives strong input from superficial cortical layers and these inputs, have been posited to contribute strongly to sensory response properties of L5 neurons (Gilbert and Wiesel, 1983; Douglas and Martin, 2004). Therefore, it is reasonable to suspect that SINs of L5 and L4 might have different response properties, and that these differences might be related to differences in thalamocortical connectivity. Indeed, we found both similarities and differences in response properties of L5 and L4 SINs, and argue that some of these differences are related to differences in thalamocortical connectivity.

RF properties of L5 vs. L4 SINs

(1) Response linearity: both L5 and L4 SINs show non-linear responses to near-optimal drifting sinusoidal gratings (F1/F0 ratios <1; Skottun et al., 1991), responding with a weakly modulated increase in firing rate. Such non-linear responding is similar to that seen in closely neighboring L5 CTect neurons (Su et al., 2024), but very different from the pronounced linear spatial summation of L4 simple cells (Zhuang et al., 2013). Classification of visual cortical neurons into “simple” or “complex” is commonly determined by the F1/F0 ratio (Movshon et al., 1978a, b; Carandini et al., 1997; Ringach et al., 2002). Thus, *nearly all of the SINs in both L4 and L5*, including all but two of the very few SINs in L5 with a single subfield, would be classified as “complex” according to this criterion.

(2) Spatial RFs: Highly overlapping ON/OFF subfields were found in *all* L4 SINs, and in the great majority (86%) of L5 SINs. However, 6 of the L5 SINs had only a single ON or OFF subfield, a RF characteristic not seen in L4 SINs.

(3) Orientation selectivity: Both L5 and L4 SINs displayed minimal directional selectivity. Orientation tuning was also relatively poor for both L5 and L4 SINs, when compared to that of simple cells of L4 (Zhuang et al., 2013) or CTect cells of L5 (Su et al., 2024). However, some L5 SINs were better tuned for stimulus orientation than any of the L4 SINs (Fig. 4F). As discussed below, we believe this is due to a different balance of thalamocortical and corticocortical inputs to these cells.

(4) Spontaneous and visually driven firing rates: In multiple sensory and motor cortical areas of awake rabbits, SINs have the highest spontaneous firing rates studied

(Swadlow, 1988, 1989, 1990, 1991, 1994). While L5 V1 SINs have high rates of spontaneous activity compared to neighboring CTect neurons in L5 (Su et al., 2024), firing rates are significantly lower in L5 than L4 SINs (Fig. 4G). Unmodulated visually driven rates (F0) of L4 and L5 SINs are very similar (Fig. 4B), but, because the modulated visually driven rates (F1) are so low in the L5 SINs, their F1/F0 ratios are also lower than in L4 SINs.

(5) Latency to visual stimulation: Response latencies to visual stimuli were significantly longer in L5 SINs than in L4 SINs (Fig. 4J). Such a laminar difference in response latencies is consistent with a “canonical cortical circuit” (Gilbert and Wiesel, 1983; Douglas and Martin, 1991, 2004) in which sensory information reaches L5 largely through an indirect pathway (thalamus to L4 to L2/3 to L5). Such a pathway would result in a longer latency to sensory stimulation in L5. Although the canonical circuit has been challenged by some studies (e.g., Constantinople and Bruno, 2013; Pluta et al., 2015), as noted above, L5 does receive fewer thalamic afferents, and more input from superficial layers than L4. Therefore, some (if not all) long latency responses mediated by superficial inputs would be expected.

RF properties of L5 SINs are related to thalamocortical inputs

Two complementary methods were used to infer thalamocortical connectivity (Swadlow and Lukatela, 1996): electrical stimulation of the LGN, and cross-correlation of retinotopically aligned LGN-SIN cell pairs. LGN stimulation will activate neurons that receive monosynaptic LGN input at a latency consistent with (a) the thalamocortical axonal conduction times (0.8 – 2 ms, for the great majority of LGN axons (Swadlow and Weyand, 1985; Stoelzel et al., 2008) plus the synaptic delay. However, LGN stimulation may be subthreshold for driving postsynaptic spikes. Moreover, thalamic stimulation can also synaptically activate cortical neurons by stimulating thalamocortical axons originating in nearby thalamic nuclei (e.g., the pulvinar, Weyand and Swadlow, 1986), or by antidromically activating corticofugal axons that pass near the thalamic stimulation site, resulting in antidromic invasion of the corticofugal axonal recurrent axon collaterals that synapse in the cortex. Therefore, while a short synaptic latency (e.g., < 3 ms) in a cortical neuron following LGN stimulation is suggestive of monosynaptic LGN input, and a longer synaptic latency is suggestive of polysynaptic input, such results are not definitive.

By contrast, cross-correlation analysis showed definitively, and for the first time, that many L5 SINs receive potent monosynaptic input from the LGN. Synaptic efficacies were in some cases extremely high; (e.g., Fig. 6A, 6C). While extracellular cross-correlation analysis cannot detect subthreshold inputs, or polysynaptic inputs, it is definitive in detection of strong monosynaptic inputs (Moore et al., 1970; Swadlow and Lukatela, 1996). Together, the methods of electrical stimulation of the thalamus, and cross-correlation of aligned LGN-SIN neuronal pairs provided results consistent with the idea that L5 SINs that display short latencies to thalamic stimulation are receiving monosynaptic input from the LGN, and have properties more similar to L4 SINs. Our

results also indicate that there is no evidence of L5 SINs with longer synaptic latencies receiving monosynaptic thalamic input. Instead, they appear to be receiving indirect (multisynaptic) LGN input, possibly mediated by superficial cortical layers. Thus, the L5 SINs responding at longer synaptic latencies to thalamic stimulation displayed greater orientation selectivity than either the short-latency L5 SINs or the L4 SINs (as do most projection cells found in L2/3, Swadlow, 1988), and responded at longer latencies to visual stimulation (also consistent with a multisynaptic pathway). Long latency L5 SINs also had lower spontaneous and visually driven firing rates, and they were deeper in L5 than were the short latency SINs. Importantly, we found that L5 SINs that received monosynaptic input from their retinotopically aligned LGN neuron (based on cross-correlation analysis) also responded to the LGN electrical stimulation at short latencies (< 2.5 ms). These LGN "connected" SINs were only found in the superficial ½ of L5 and had spontaneous firing rates considerably higher than those of non-connected SINs (C vs. NC in Fig. 7).

Conclusions

Despite the very different morphology and connectivity of L4 and L5 in mammalian neocortex, L5 SINs display remarkable similarities with SINs of L4, including response non-linearity, largely overlapping ON/OFF spatial subfields, and a wide variety of visual response properties. L5 SINs also differed in a number of respects from L4 SINs, and some of these differences are related to the presence of monosynaptic LGN inputs that we demonstrate in L5 SINs for the first time. Investigating the role of thalamocortical (and other) inputs in the synthesis of sensory response properties of these neurons is crucial to understand the functional diversity of fast-spike inhibitory interneurons in different layers of sensory neocortex.

Figure captions

Figure 1. General methods. A, Schematic of stimulating and recording electrode placement in experimental preparation. Spikes were recorded from LGN neurons and from cortical L5 SINs. The same cortical electrode used to record the L5 SIN spikes was used to record local field potentials and to compute spike-triggered LFPs (st-LFPs) elicited by LGN spikes (Jin et al., 2008; Swadlow and Gusev, 2000; Stoelzel et al., 2008). Stimulation electrodes were implanted in LGN to identify SINs. B, SINs were identified with at least 3 spikes with minimum interspike interval (ISI) < 1.667 ms to electrical stimulation. C, Distribution of the spike waveform duration of the L5 SINs, L4 SINs (from Zhuang et al., 2013) and CTect neurons (from Su et al., 2024), measuring from trough to peak. D, An example of cross-correlogram between an LGN neuron and an L5 SIN. The depth of L5 SIN recording site, efficacy (E), and contribution (C) are shown on the upper left of the cross-correlogram. The retinotopic alignment between the LGN neuron with an OFF-center concentric RF (blue oval contour shows the center

position) and the L5 SIN RFs (red: ON response, blue: OFF response) are shown on the left side of the cross-correlogram as well.

Figure 2. Spatial RF structures of the L5 SINs. A1, The majority (86%) of L5 SINs have highly overlapping ON-OFF subfields. Left, heat maps for SIN responses to light stimuli (for ON subfield) and dark stimuli (for OFF subfield). Right, spatial RF map for the cell. Red, ON subfield; Blue, OFF subfield. A2-A3, A few L5 SINs have a single ON or OFF subfield. B, Distribution of Sign Index. The majority of L5 SINs have Sign Index values close to 0, indicating balanced ON and OFF subfields. Two SINs have Sign Index < -0.7, and therefore were classified as having a single OFF subfield. Four SINs have Sign Index > 0.7 and were classified as having a single ON subfield. C, Distribution of Local Similarity Index (LSI) of L5 SINs with both an ON and an OFF subfield. LSI was not measured for the SINs with a single subfield. The L5 SINs have highly overlapped ON and OFF subfields. The red and blue circles illustrate ON and OFF subfields are completely separated when LSI = 0 or are completely overlapped when LSI = 1. For those six L5 SINs with a single subfield, their LSIs were not calculated.

Figure 3. L5 SINs, like SINs of L4, respond in a nonlinear manner to a near-optimal drifting visual grating. A, An example of an L5 SIN responding to its optimal drifting sinusoidal grating in a nonlinear manner with a F1/F0 ratio < 1. B, An example of an L4 simple cell responding to its optimal drifting sinusoidal grating in a linear manner, with F1/F0 ratio > 1. C, Distributions of F1/F0 ratio for L5 SINs (upper panel) and L4 simple cells (lower panel). All but two of the L5 SINs have F1/F0 ratio < 1, thus they are considered to have unmodulated response and can be classified as complex cells according to their nonlinear responses (Movshon et al., 1978a; Skottun et al., 1991). By contrast, the great majority of L4 simple cells (as defined by their spatial RF structure, Zhuang et al., 2013) have an F1/F0 ratio > 1, thus they have a modulated response and can also be classified as simple cells according to their linear responses (Movshon et al., 1978b; Skottun et al., 1991).

Figure 4. Similarities (A-E) and differences (F-L) between L5 SINs and previously studied L4 SINs: A, Distributions of LSI for L5 SINs and L4 SINs. High LSI values mean greater overlap between ON and OFF subfields. B, Distributions of maximum evoked F0 responses for L5 SINs and L4 SINs measured with their optimal drifting grating stimulation. C, Distribution of Fano Factor for L5 SINs and L4 SINs. Fano Factor was used to measure the respond reliability of the cells. Fano factors are lowest in cells that respond most reliably to visual stimuli. D, Distribution of directional selective index (DSI) for L5 SINs and L4 SINs. Both L5 and L4 SINs have low values of DSI, indicating poor selectivity for stimulus direction. E, Distribution of sustained index for L5 and L4 SINs.

Figure 5. Synaptic latency to LGN stimulation is related to visual properties. A, Distribution of synaptic latency for all L5 SINs. B, The depth of the L5 SINs is loosely correlated with their synaptic latency. Black dots represent individual L5 SINs, while four red dots indicate average depths for SINs with latencies ranging from 1-2 ms, 2-3 ms, 3-4 ms, and 4-5 ms, with vertical red lines indicating the standard error of the mean for the

average depths. The cells located deeper in the L5 tend to have longer synaptic latency. The label indicating Pearson's r = 0.286 and p-value = 0.021 is based on the raw data (represented by black dots). While Pearson's r = 0.981 and p-value = 0.019 are based on the averaged depths for SINs across different synaptic latency ranges (red dots, r and p values are not shown on the figure). C, The synaptic latencies of L5 SINs are correlated with orientation selectivity, SINs with longer synaptic latency are more tuned for stimulus orientation. D, The synaptic latencies of L5 SINs to electrical stimulation of the LGN are correlated with visual latencies to flash stimulation. SINs with longer synaptic latency respond at longer latencies to visual flash stimulation. E – F, The synaptic latencies of L5 SINs to electrical stimulation of the LGN are correlated with the spontaneous and evoked F0 activities. SINs with longer synaptic latency have lower spontaneous activity and lower evoked F0 activity.

Figure 6. L5 SINs received monosynaptic LGN inputs. A-M, LGN-L5 SIN cross-correlograms organized in descending sequence of their efficacy values. Retinotopic alignment between LGN and L5 SINs RF maps are showed above each cross-correlogram. The horizontal bars below the RF maps represent a length of 2 degrees. Each of the SINs is labeled with respect to the reversal point of the flash-evoked potential. Efficacy (E) and contribution (C) of each pair connection are listed below their RFs. Three SINs received convergent input from two different LGN neurons. A-B, An L5 SIN received convergent inputs from one OFF concentric LGN neuron (A), and one ON concentric LGN neuron (B). C-D, Another L5 SIN received convergent inputs from one OFF concentric LGN neuron (C), and one ON concentric LGN neuron (D). G-H, A third SIN received convergent inputs from one OFF concentric LGN neuron (G), and one ON concentric LGN neuron (H). N, Depth distribution of L5 SINs recorded relative to the location of CTect neurons (N, right column). The SINs (solid dots) were recorded either simultaneously with a CTect neuron, sequentially within 60 µm of a CTect neuron, or ~ 200 µm below a CTect neuron using the same electrode during a single penetration. Three SINs (open dots) were not recorded simultaneously with a CTect neuron. The depth of the CTect neurons recorded with these seven SINs is shown in the left column (green dots).

Figure 7. Differences between the L5 SINs receiving monosynaptic LGN inputs versus those, with similar retinotopic alignment, that do not receive monosynaptic LGN inputs. A, The connected L5 SINs (C) were located more superficially than those L5 SINs that did not receive monosynaptic LGN input (NC). B, The spike-triggered LFPs near the connected L5 SINs (recorded through the same microelectrode) were significantly stronger than the spike-triggered LFPs near the L5 SINs that did not receive monosynaptic LGN input. C, The connected L5 SINs have significantly higher spontaneous activity than the non-connected L5 SINs. D, The connected L5 SINs all responded very fast to thalamic electrical stimulation compared to the non-connected L5 SINs.

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866 Table 1. Cell numbers of L5 SINs

RF types	Number of cells	% of whole population
ON-OFF overlapped	56	86.15%
Single ON	4	6.15%
Single OFF	2	3.08%
Suppressive	2	3.08%
Not responding	1	1.54%
Total	65	100%

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