Translaminar recurrence from layer 5 suppresses superficial cortical layers

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Information flow in the sensory cortex has been described as a predominantly feedforward sequence with deep layers as the output structure. Although recurrent excitatory projections from layer 5 (L5) to superficial L2/3 have been identified by anatomical and physiological studies, their functional impact on sensory processing remains unclear. Here, we use layer-selective optogenetic manipulations in the primary auditory cortex to demonstrate that feedback inputs from L5 suppress the activity of superficial layers regardless of the arousal level, contrary to the prediction from their excitatory connectivity. This suppressive effect is predominantly mediated by translaminar circuitry through intratelencephalic neurons, with an additional contribution of subcortical projections by pyramidal tract neurons. Furthermore, L5 activation sharpened tone-evoked responses of superficial layers in both frequency and time domains, indicating its impact on cortical spectro-temporal integration. Together, our findings establish a translaminar inhibitory recurrence from deep layers that sharpens feature selectivity in superficial cortical layers.
 Revealing the principles governing interactions between the six layers of the cortex is fundamental for understanding how these intricately woven circuits work together to process sensory information. In the canonical cortical circuit model, information flow in the cortex has been considered as a predominantly feedforward sequence along the L4 → L2/3 → L5/6 axis, from which information is sent out to subcortical structures. However, the cortex is not simply a feedforward circuit but is full of recurrent circuits, the activity of even a single neuron can be transmitted back to itself, resulting in non-linear changes in the entire network. Recent studies have started to shed light on the computational consequences of local recurrence within a single cortical layer and have demonstrated its roles in shaping cortical sensory tuning and its feedback projections onto superficial layers could potentially expand the computational capacity of cortical circuits.

L5 of the sensory cortex is considered an output hub that sends long-range projections to a variety of other cortical and subcortical structures. L5 pyramidal cells are classified into two large categories: intralaminar (IT) neurons, whose axons stay within the cortex and striatum, and pyramidal tract (PT) neurons, which project to multiple subcortical structures, including the thalamus. Importantly, IT neurons, and a subset of PT neurons, send their axons to superficial layers within the cortical column in addition to their distal targets, providing a potential substrate for translaminar recurrence between L2/3 and L5. Indeed, this excitatory feedback projection from L5 pyramidal cells to L2/3 neurons has been confirmed by extensive anatomical and electrophysiological studies in vitro and in vivo. The functional impact of this excitatory feedback projection remains controversial in vivo. One study reported that activation of L5 pyramidal cells depolarized L2/3 neurons and triggered transitions to the global up state under anesthesia, supporting the feedback excitatory circuitry that enhances and prolongs sustained activity within the cortical column. In contrast, recent work combining two-photon calcium imaging and targeted optogenetics in awake mice found sparse activation of L5 neurons to be ineffective in driving L2/3 neurons, whereas activation of L2/3 neurons robustly recruited L5 neurons. In agreement with the latter finding, L5 pyramidal cells also send projections to L2/3 inhibitory neurons, which may counteract the excitatory effects of L5 neurons onto superficial layers. Therefore, it remains unclear how the interplay between excitatory and inhibitory pathways shapes the net functional impact of L5 neurons on sensory processing in superficial layers.

Here, by combining bidirectional optogenetic manipulations of L5 neurons with single-unit recordings across the entire cortical column in the primary auditory cortex (A1), we find the unexpected result that L5 activity in awake mice suppresses both spontaneous and tone-evoked activity in superficial layers. Thus, the ascending projection of L5 neurons forms an inhibitory recurrence, which sharpens tone-evoked responses of superficial layers in both the frequency and time domains. Recordings from the auditory thalamus and selective manipulations of L5 IT and PT subpopulations both indicate that this suppression is mediated by the coordinated action of intracortical and subcortical projections, with a substantial contribution of the intracortical inhibitory circuit. These results add a missing piece to existing cortical circuit models and reveal a critical role of translaminar recurrent circuits in shaping cortical sensory representations.

**Results**

Optogenetic activation of L5 suppresses superficial cortical layers. To determine how L5 activity impacts sensory processing in other cortical layers, we conducted linear probe recordings across the entire cortical column while optogenetically manipulating L5 neurons in A1 of awake mice (Fig. 1a–c). We expressed the red light-sensitive cation channel ChrismomR34 in L5 pyramidal cells by injecting a Cre-dependent adeno-associated virus (AAV) vector in Rbp4-Cre mice. In this strain, transgene expression is restricted to excitatory neurons in both superficial and deep sublayers of L531,33,36 with dense neuropil in superficial layers (Fig. 1b). Three weeks after targeted AAV injections in A1 mapped with intrinsic signal imaging (Fig. 1d), recordings were conducted near the injection site by inserting a 64-channel linear probe perpendicular to the cortical surface. We recorded unit activity simultaneously from all six layers in awake head-fixed mice and identified L4 boundaries as the early sink in current source density analysis (Fig. 1e). Illumination of the A1 cortical surface with red LED (625 nm) triggered a rapid and reversible increase in the spontaneous firing of L5 regular-spiking (RS) units, demonstrating the effectiveness of our ChrismomR-mediated photoactivation (Fig. 1f, g).

The prediction from the well-accepted L5 → L2/3 → L5/6 projection connection is that activation of L5 will recruit L2/3 pyramidal cells into enhanced recurrent activity. To test this model, we first focused on RS units, which primarily represent excitatory neurons, and quantified the effect of L5 photoactivation by computing a modulation index (MI) for multi-unit firing at depth bins along the cortical column (Fig. 1g). MI was calculated as (L – C)/(L + C), where L represents the activity in LED trials and C represents the activity during No LED control trials. Thus, MI ranges from -1 to 1, where a value of -1 represents a complete loss of activity, 1 represents the emergence of activity from nothing, and 0 represents no change. In contrast to our prediction, optogenetic L5 activation strongly suppressed spontaneous firing of RS units throughout the cortical column. Calculation of MI for isolated RS single-units demonstrated predominant suppression in both L2/3 and L4, while the effect on L6 was more variable (91%, 89%, and 63% of units showed suppression in L2/3, L4, and L6; L2/3: p = 2.4 × 10^{-11}; L4: p = 5.4 × 10^{-10}; L6: p = 6.3 × 10^{-5}, Wilcoxon signed rank test; Fig. 1h, i, and Supplementary Fig. 1a). We observed only a small fraction of activated units, which mostly resided in L6 (6%, 5%, 27% of units in L2/3, L4, and L6; Supplementary Fig. 1a). Notably, suppression was stronger in L2/3 than L4 (p = 0.016, Wilcoxon rank sum test; Fig. 1i), suggesting that L2/3 does not simply inherit its suppression from L4. Thus, these data show that stimulation of L5 excitatory neurons suppresses activity in the superficial layers of A1.

A potential concern of photostimulation results is that the artificial L5 activity patterns may modulate cortical circuits differently from endogenous L5 activity. To address this issue and determine the impact of L5 activity on other layers in physiological conditions, we next performed loss-of-function experiments. We virally expressed inhibitory opsin eNPHR3.0 in L5 pyramidal cells using Rbp4-Cre mice. Illumination of the cortical surface with amber LED (595 nm) rapidly suppressed L5 RS unit activity in A1 (Fig. 1j, k). Consistent with our L5 photoactivation results, L5 inactivation enhanced the spontaneous firing of RS units throughout the cortical layers (Fig. 1k).

MI for RS single-units demonstrated predominant activation of L2/3 and L4, with more variable effects on L6 (79%, 75%, and 52% of units showed enhancement in L2/3, L4, and L6; L2/3: p = 6.1 × 10^{-8}; L4: p = 9.3 × 10^{-2}; L6: p = 0.13, Wilcoxon signed rank test; Fig. 1l, m, and Supplementary Fig. 1b). Again, the...
The enhancement of activity was more pronounced in L2/3 than L4 (p = 0.018, Wilcoxon rank sum test; Fig. 1m), suggesting a site of suppression within L2/3. Collectively, our bidirectional manipulations demonstrate that both photoevoked and endogenous L5 activities have suppressive effects on superficial cortical layers in A1 of awake mice.

Our finding of L5-mediated negative regulation onto superficial layers contrasts with a previous study that reported L5 activation in anesthetized mice triggered a transition to cortical up state and increased L2/3 activity. We wondered if this discrepancy could be due to different cortical circuit operations across brain states in recorded animals. To test the robustness of
our results across brain states, we examined if the arousal level of awake mice influenced the suppressive effects of L5 onto other layers. Throughout A1 unit recordings and L5 optogenetic manipulations, we monitored pupil diameter, a reliable readout for the animal’s arousal level\(^37\,\,38\) (Fig. 2a). We used wideband LED intensities to prevent activity from reaching a floor or ceiling. As reported previously\(^37\), L2/3 RS multi-unit spontaneous firing exhibited a U-shaped relationship with pupil size in the control condition (Fig. 2b). Bidirectional optogenetic manipulation of L5 activity scaled L2/3 firing rate while maintaining its U-shaped relationship with the pupil diameter, resulting in comparable MI values across arousal levels (L5 activation: \(p = 0.96\); L5 inactivation: \(p = 0.43\); one-way ANOVA; Fig. 2b±e). These results demonstrate that the suppressive effect of L5 onto superficial layers is robust regardless of the arousal level.

We next directly compared the effects of L5 optogenetic manipulations before and after inducing urethane anesthesia within the same mice (Fig. 2f). Similar to our results in the awake state, calculation of MI showed that L5 activation under anesthesia strongly suppressed spontaneous firing in superficial layers (96%, 80%, and 58% of single-units showed suppression in L2/3, L4, and L6; L2/3: \(p = 8.4 \times 10^{-5}\); L4: \(p = 3.7 \times 10^{-4}\); L6: \(p = 0.55\), Wilcoxon signed rank test; Fig. 2g, h, and Supplementary Fig. 1c). The strength of suppression in L2/3–L4 and the increase of L5 firing were not different between the two states (L2/3–L4: \(p = 0.67\); L5: \(p = 0.45\), paired t-test; Fig. 2i). Similarly, L5 inactivation facilitated the activity of superficial layers under anesthesia (73%, 71%, and 68% of single-units showed enhancement in L2/3, L4, and L6; L2/3: \(p = 0.018\); L4: \(p = 0.0067\); L6: \(p = 7.0 \times 10^{-4}\), Fig. 2j, k, and Supplementary Fig. 1d), and the strength of modulation was equivalent between the awake and anesthetized states (L2/3–L4: \(p = 0.91\); L5: \(p = 0.55\); Fig. 2l). We also confirmed that the use of constant-intensity LED without a ramp, similar to the one used in the previous study, or the use of weaker LED intensities did not affect our conclusions (Supplementary Fig. 2). Taken together, L5 activity suppresses superficial cortical layers regardless of brain state, indicating continuous sparing of L2/3 neural activity by negative feedback from L5.

L5 sharpens sensory tuning of superficial layers in both frequency and time domains. How does the suppressive effect of L5 shape sound representations in superficial cortical layers? To address this question, we determined whether L5 activation alters frequency tuning of A1 neurons across the depth of the cortical column. We generated isointensity functions of RS single-units by presenting pure tones across a range of frequencies (70 dB SPL, 4–64 kHz, 0.2 s), alternating control trials with L5 photostimulation trials (Fig. 3a). On top of the suppression of spontaneous firing, L5 activation attenuated tone-evoked activity in the superficial layers (Fig. 3a, b). Calculation of MI for tone-evoked activity showed that most RS single-units in L2/3 and L4 decreased their response amplitudes, while the effect on L6 was variable (78%, 67%, and 46% of unit-frequency pairs showed suppression in L2/3, L4, and L6; L2/3: \(p = 3.4 \times 10^{-9}\); L4: \(p = 4.9 \times 10^{-6}\); L6: \(p = 0.12\), Wilcoxon signed rank test; Fig. 3c and Supplementary Fig. 3a). Similar to the impact on spontaneous activity, the suppression of tone responses was more robust in L2/3 than in L4 (\(p = 0.035\), Wilcoxon rank sum test). Comparison of the best frequency (BF) between control and LED trials showed that L5 photostimulation did not affect preferred frequency in other layers (Supplementary Fig. 3b). However, the overall suppression of tone-evoked activity narrowed the frequency tuning bandwidth in L2/3 and L4 (L2/3: \(p = 1.7 \times 10^{-4}\); L4: \(p = 0.018\); L6: \(p = 0.16\), Wilcoxon signed rank test; Fig. 3d), indicating the sharpening of frequency tuning in superficial layers.

To understand the transformation underlying L5-mediated sharpening of frequency tuning in L2/3–L4 neurons, we distinguished between subtractive and divisive mechanisms by plotting tone-evoked responses under control versus L5 photostimulation conditions (Fig. 3e)\(^39\,\,41\). By applying threshold-linear fit to the data points, we determined whether suppression in individual single-units was subtractive (y-intercept far from zero), divisive (slope far from one), or a combination of both (Methods; Supplementary Fig. 4). We found that suppression in L2/3–L4 was a mixture of subtractive and divisive control, with the latter being dominant (Fig. 3f, g): approximately half (42%) of units showed only divisive suppression, 9% showed only subtractive suppression, and 15% showed both mechanisms. This result is distinct from the purely divisive scaling found in L6-dependent suppression in the primary visual cortex (V1)\(^42\) but consistent with the mixed mechanisms of gain regulation by A1 cortical inhibitory neuron activation\(^39\).

In L5 inactivation experiments, we observed the overall inverse of the L5 activation results, except for less pronounced effects on L4 (see Discussion). Calculation of MI showed that most RS single-units in L2/3 increased their tone-evoked responses, while L4 and L6 results were variable (57%, 42%, and 24% of unit-frequency pairs showed enhancement in L2/3, L4, and L6; L2/3: \(p = 9.1 \times 10^{-11}\); L4: \(p = 0.20\); L6: \(p = 0.51\), Wilcoxon signed rank test; L2/3 vs. L4: \(p = 0.0040\), Wilcoxon rank sum test; Fig. 3h–j and Supplementary Fig. 3c). The overall enhancement of tone-
evoked activity broadened the tuning of L2/3 units without affecting their preferred frequency (L2/3: p = 0.0021; L4: p = 1.0; L6: p = 1.0), Wilcoxon signed rank test; Fig. 3k and Supplementary Fig. 3d). Application of threshold-linear fitting to each tone-responsive unit showed that the impact of L5 inactivation on superficial layers was a mixture of additive and multiplicative transformations, with the latter being slightly more dominant (Fig. 3l–n). Together, our results from bidirectional manipulations of L5 revealed that both photoevoked and endogenous activity of L5 sharpens frequency tuning in superficial cortical layers. The mixed divisive and subtractive transformations may indicate cortical inhibitory mechanisms in L5-dependent suppressive gain control of superficial layers.

Recurrent excitatory circuits are considered to provide neural substrates for maintaining sustained activity.

43 Our unexpected finding of inhibitory recurrence from L5 onto L2/3 thus raises a question—does L5 activity prolong13 or truncate44 sensory activity in superficial layers? To address this question, we examined the impacts of L5 activation and inactivation on three kinetics parameters: response latency, decay time, and full-width of half-maximum (FWHM) of tone-evoked responses in L2/3–L4 multi-unit activity (Fig. 4a,e). While we found no change in response latency with either manipulation (activation: p = 0.13; inactivation: p = 0.10, paired t-test, Fig. 4b, f), L5 activation and inactivation shortened and prolonged decay time, respectively (activation: p = 0.074; inactivation: p = 0.0027, Fig. 4c, g).
Consequently, we observed FWHM truncation in L5 activation ($p = 0.016$; Fig. 4d) and elongation in L5 inactivation ($p = 0.020$; Fig. 4h). Therefore, L5 activity sharpens the timing of tone-evoked responses in superficial layers, further supporting inhibitory rather than excitatory recurrence of L5 → L2/3 circuitry. Together, these results demonstrate that L5 sharpens sound responses of superficial layers in both the spectral and temporal dimensions, suggesting its impact on cortical spectro-temporal integration.

Intracortical and subcortical pathways for L5 inhibitory recurrence. Two independent pathways could mediate L5-dependent suppression of superficial cortical layers. On one hand, intracortical projections of L5 neurons could recruit local inhibitory neurons to suppress superficial layers. On the other hand, L5 activity could indirectly suppress the primary auditory thalamus (ventral part of the medial geniculate nucleus: MGv), which may in turn reduce feedforward excitation onto A1 L4. In our optogenetic manipulation, the suppressive effect of L5 was consistently larger in L2/3 than its upstream L4 (Fig. 1i, m; Fig. 3c, j), indicating the role of intracortical mechanisms. To examine if there is an additional contribution of subcortical pathways, we evaluated the relative impacts of both mechanisms.
by conducting unit recordings in A1 and MGv in the same mice during L5 manipulation (Fig. 5a). By inserting a linear probe deeper after A1 recordings, we were able to reach MGv, where we observed time-locked click sound responses (Fig. 5b, c). We found that L5 optogenetic activation reduced spontaneous spikes in MGv, but to a lesser extent than in A1 L2/3–L4 (MGv: 40.2% reduction; L2/3-L4: 59.9%; p = 0.033, paired t-test; Fig. 5d, e). In the L5 inactivation experiments, the difference of modulation magnitudes between MGv and A1 superficial layers was more prominent; L5 inactivation only slightly increased spontaneous spikes in MGv, whereas A1 L2/3–L4 activity showed four-times larger enhancement in the same mice (MGv: 19.2% enhancement; L2/3–L4: 79.5%; p = 0.028, paired t-test; Fig. 5f, g). These results indicate the involvement of both intracortical and subcortical pathways in L5-dependent suppression of superficial cortical layers, although the endogenous activity of L5 acts predominantly through intracortical mechanisms. In a separate set of mice, we also performed unit recordings in the inferior colliculus. Consistent with a previous study, L5 activation triggered a small enhancement of spontaneous firing in the external cortex, but not the central nucleus of the inferior colliculus (Supplementary Fig. 5). The lack of effect on the lemniscal inferior colliculus
suggests that the L5-dependent small suppression of MGv is not inherited from upstream and arises at the level of the thalamus.

Intracortical and subcortical mechanisms of L5-dependent inhibitory recurrence should suppress the superficial layers with distinct kinetics. To test this prediction, we combined multi-unit spikes during L5 optogenetic activation across mice and compared the suppression kinetics between A1 L2/3, L4, and MGv. Note that we excluded L2/3–L4 units with increased spikes to clarify the suppression onset; however, the incomplete removal of activated units likely caused an overestimation of suppression latency in these layers. Despite this overestimation, we found that the suppression onset for L2/3 was earlier than L4 and comparable to MGv (9.0, 18.5, and 9.1 ms latency in L2/3, L4, and MGv; Fig. 5h), indicating that MGv suppression was not fast enough to account for the short-latency suppression in L2/3. Indeed, the L2/3 spike rate was fit by the sum of two exponential curves, which likely corresponds to fast intracortical and slow subcortical mechanisms, while L4 and MGv data were fit better by a single exponential. Quantification of suppression magnitudes explained by the first and second exponentials revealed that the fast component accounted for 63% of total suppression in L2/3 (fast component: 0.47; slow component: 0.28). Together, these data provide further evidence that L5 activity suppresses L2/3 neurons predominantly through the intracortical pathway.

**IT neurons modulate superficial layers more robustly than PT neurons.** Our results revealed the contribution of both intracortical and subcortical pathways in L5-mediated inhibitory recurrence onto superficial cortical layers. Based on this observation, we next asked if there are distinct roles for the two large categories of L5 pyramidal cells: IT neurons, which predominantly contribute to intracortical projections, and PT neurons, which are the sole source of subcortical projections (Fig. 6a). To address this question, we selectively manipulated L5 IT and PT neurons using a combination of viral approaches. Since IT neurons connect to PT neurons while PT neurons rarely synapse onto IT neurons, the difference in the effects between the two manipulations is likely attributed to intracortical projections of IT neurons.
We selectively expressed optogenetic tools in IT neurons using Tlx3-Cre mice (Fig. 6b). PT neurons were retrogradely labeled by injection of CAV2-Cre into the inferior colliculus. By expressing synaptophysin-EYFP using these strategies, we confirmed distinct distribution of their somata across the cortical depth (IT neurons in L5a and PT neurons in L5b; Fig. 6c) and their differential projections onto subcortical structures (Supplementary Fig. 6).

To determine the difference between the effects of IT and PT activation, we first expressed ChrimsonR selectively in each subtype and conducted photostimulation during A1 unit recordings. Illumination of the cortical surface with red LED
triggered an increase in spontaneous firing at expected cortical depths (Fig. 6d, e), and the magnitudes of L5 activation were comparable between IT and PT manipulations (Supplementary Fig. 7). Calculation of MI for RS single-units demonstrated two noticeable differences between IT and PT photostimulation. First, although both IT and PT activation suppressed L4 spontaneous activity to a comparable level (IT vs. PT: p = 0.10, Wilcoxon rank sum test), IT activation triggered significantly larger suppression in L2/3 (IT vs. PT: p = 0.0021; Fig. 6f and Supplementary Fig. 8), further supporting the suppression of L2/3 by the L5 intracortical projections. PT activation additionally suppressed L6, which was absent in IT activation (IT vs. PT: p = 0.014). Second, MI for tone-evoked activity demonstrated that only IT, not PT, activation suppressed tone responses in superficial layers (L2/3: p = 1.3 × 10−4; L4: p = 0.040, Wilcoxon signed rank test; Fig. 6g). Indeed, the sharpening of frequency tuning in superficial layers was comparable between pan-L5 neuron activation and IT neuron activation alone (Fig. 3 and Supplementary Fig. 9). Therefore, intracortical recurrence from IT neurons plays a crucial role in regulating both spontaneous and tone-evoked activity of superficial layers. In contrast, subcortical projections from PT neurons suppress only spontaneous firing and do not affect tone-evoked activity.

We next expressed eNpHR3.0 selectively in IT or PT neurons and conducted subtype-specific loss-of-function experiments (Fig. 6h, i). Surprisingly, although the magnitudes of L5 inactivation were again comparable between IT and PT manipulations (Supplementary Fig. 7), quantification of MI for RS single-units revealed a striking difference. Inactivation of IT neurons enhanced both spontaneous and tone-evoked activity in L2/3 (spontaneous: p = 2.9 × 10−4; tone-evoked: p = 4.1 × 10−4, Wilcoxon signed rank test; Fig. 6j, k), similar to pan-L5 inactivation using Rbp4-Cre mice (Fig. 1m, 3j, and Supplementary Fig. 9). In contrast, PT inactivation did not cause any change in the firing of other layers. The lack of effects by PT photoinactivation agrees with our observation that pan-L5 inactivation minimally affected MGv activity (Fig. 5f, g), which both indicate that the endogenous level of L5 activity is insufficient in suppressing subcortical structures. Taken together, our results demonstrate that intracortical projections of IT neurons predominantly mediate the suppressive effects of L5 onto superficial layers. Subcortical projections of PT neurons likely provide an additional layer of suppression only in the face of substantially raised activity.

Finally, we investigated the inhibitory mechanisms by which intracortical L5 recurrence negatively regulates superficial layers. To test whether changes in inhibitory neuron activity account for these suppressive effects, we combined data from pan-L5 (Fig. 1) and IT neuron (Fig. 6) manipulation experiments and analyzed fast-spiking (FS) units, which mostly represent parvalbumin-expressing inhibitory neurons. Interestingly, optogenetic activation of L5 neurons suppressed FS single-units in L2/3–L4 while those in L5–L6 were variable but overall unchanged (Fig. 7a, b). Therefore, the suppressive effects of L5 cannot be simply explained by the increased activity of inhibitory neurons. However, focusing on spike changes at the LED onset, we found many L2/3–L4 FS units that transiently increased their firing before switching to suppression (Fig. 7c, d). Combining transient and sustained activation, we found a three times higher fraction of FS single-units showing LED-driven excitation than RS single-units (RS: 11.2%; FS: 39.2%; p = 0.0015; Fisher exact test). The biphasic modulation of inhibitory neurons and co-suppression of both excitatory and inhibitory neurons are in agreement with the recently observed rebalancing of cortical circuits after optogenetic manipulation of inhibitory neurons in awake mice.6,7,16,17 These theoretical studies have contributed to the dynamics of the local recurrent network (inhibition-stabilized network model; Supplementary Fig. 10).4,8,47 Conversely, optogenetic inactivation of L5 neurons increased the firing of L2/3–L4 FS units without affecting those in L5–L6 (Fig. 7e, f). We observed biphasic modulations of L2/3–L4 FS units at the LED onset and found a three times higher fraction of FS single-units showing LED-driven suppression than RS units (RS: 9.7%; FS: 30.0%; p = 0.016; Fisher exact test; Fig. 7h), which again agrees with circuit rebalancing. Therefore, our data are most consistent with a model where L5 IT neurons send ascending projections that preferentially synapse onto FS inhibitory neurons in superficial layers, although we do not exclude the contribution of other inhibitory neuron subtypes.

Discussion

Dense recurrent networks, including feedforward, feedback, and lateral projections, are a hallmark of mammalian cortical circuits and are considered to underlie higher integrative functions, such as working memory and consciousness.13,48–50 Both anatomical and electrophysiological studies have found that recurrent circuits provide the majority of sensory-evoked excitation to cortical neurons.2,11,51,52 Therefore, understanding cortical computations critically relies on our knowledge of the functions of recurrent circuits. While extensive previous work has described the mutual excitatory projections between L2/3 and L5 pyramidal cells in many cortical areas, the functional consequence of translaminar recurrent circuits is more elusive. In this study, we took advantage of genetic and viral tools to selectively manipulate L5 pyramidal...
cell subpopulations and found an unexpected suppression of superficial layers by L5 activity in both awake and anesthetized animals. Our results, therefore, reveal a key principle of cortical circuit operation; translaminar circuitry allows the output layer of the cortex to provide negative feedback to its upstream target, which constantly sparsens the activity of neurons in superficial layers and sharpens their tuning in sensory space.

Our results identified L5-mediated suppression of superficial layers via both intracortical and subcortical pathways. Three lines of evidence support the substantial role of the intracortical

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Fig. 7 L5 recurrent inputs preferentially recruit fast-spiking units in superficial layers. a–d Data for fast-spiking (FS) units during L5 activation with ChrimsonR, combining data from Rbp4-Cre and Tlx3-Cre mice. a Laminar profile of MI for the spontaneous firing of FS multi-units during L5 activation overlaid with individual data points (n = 16 mice). Depth is normalized in each mouse according to the surface, L4 borders, and white matter positions. Red shade indicates L5. Larger bins were used compared to RS units data due to the sparsity of FS units. Results are mean ± SEM.

b Scatter plot showing MI for the spontaneous firing of FS single-units across mice (n = 23 and 40 for L2/3–L4 and L5–L6). Red dots and bars represent median and 25th and 75th percentiles. **p < 0.01. Individual groups: two-sided Wilcoxon signed rank test with Bonferroni correction (black asterisks). Comparisons across groups: two-sided Wilcoxon rank sum test (brown). c Raster and PSTH of representative L2/3 FS single-units showing sustained (left) or transient (right) enhancement of spontaneous firing during L5 activation.

d Fraction of L2/3-L4 RS and FS single-units showing sustained enhancement (pink), transient enhancement (green), suppression (blue), and no change (gray) (16 mice; RS, n = 205 units; FS, n = 23 units). **p = 0.0015 (two-sided Fisher exact test).

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e–h Same as in a–d but for L5 inactivation with eNpHR3. e n = 19 mice. f n = 20 and 48 for L2/3-L4 and L5–L6. g Raster and PSTH of representative FS single-units showing sustained (left) or transient (right) suppression of spontaneous firing during L5 inactivation.

e–h Fraction of L2/3-L4 RS and FS single-units showing sustained (pink), transient (green), suppression (blue), and unchanged (gray) responses (16 mice; RS, n = 205 units; FS, n = 23 units). *p = 0.016 (two-sided Fisher exact test).

i Schematic illustrating the translaminar inhibitory feedback from L5 that sharpens L2/3 sound-evoked responses in both frequency and time domains. See Supplementary Data 1 for additional statistics. Source data are provided as a Source Data file.
translaminar circuit: first, the effects of L5 manipulations were consistently the largest in L2/3, which were followed by L4 and MGv. Second, despite our likely overestimation of the onset latency for LED-triggered suppression, the latency for L2/3 suppression was no longer than that of MGv. Lastly, manipulations of IT neurons had more robust effects on L2/3 than the same manipulations of PT neurons.

Although the exact synaptic mechanisms underlying intracortical suppression remain to be identified, our data show that L5 activation preferentially recruits FS neurons over RS neurons by threefold in superficial layers. This result is consistent with L5 pyramidal cell projections to L2/3 FS neurons and small-basket-like adapting interneurons in vitro. It may appear at odds that both RS and FS units show suppression at the steady state during L5 activation. However, this co-modulation of excitatory and inhibitory neurons is explained by the recurrent network dynamics in an inhibition-stabilized network, as experimentally demonstrated in multiple cortical areas in awake animals. Indeed, the kinetics of transiently activated L2/3 FS units is consistent with this model and supports the role of L5 excitatory inputs onto L2/3 inhibitory neurons. Nonetheless, our data do not exclude the contribution from other pathways involving inhibitory neurons in both deep and superficial layers. For example, L5 Martinotti cells and L6 basket cells inhibit pyramidal cells in L2/3, and somatostatin-expressing L5 non-Martinotti cells target L4 for inhibition. Furthermore, since L2/3 somatostatin-expressing neurons play critical roles in regulating cortical tuning, their recruitment may also contribute to the L5-mediated inhibitory feedback. The existence of multiple inhibitory pathways may ensure the robust feedback suppression of superficial layers in the face of various patterns of L5 activities.

Historically, excitatory feedback projections between L5 and L2/3 pyramidal cells have been reported by numerous studies. However, their estimated connectivity is relatively low, which likely accounts for the lack of observed L5 → L2/3 connectivity in other studies. Moreover, L5 pyramidal cells also send projections to L2/3 inhibitory neurons, making it difficult to estimate the net impact of this translaminar recurrence in vivo. In our L5-selective optogenetic activation, we observed a transient or sustained increase of firing in 11% of RS units in superficial layers, in agreement with sparse excitatory connectivity of the ascending projection. Importantly, however, the fraction of activated FS units was three times higher (39%) in superficial layers, explaining the net inhibitory impact of L5 activation on L2/3 firing in vivo. Although optogenetic activation experiments need interpretation with caution due to potentially non-physiological activity patterns, our eNHR3-mediated loss-of-function experiments provide strong evidence that L5 suppresses L2/3 activity in physiological conditions.

Our results are consistent with a recent study that reported poor recruitment of L2/3 neurons by sparse L5 activation. We conceptually extend this finding by further demonstrating the net suppression of L2/3 firing, which was not observed in the previous study due to their use of calcium imaging. Surprisingly, one previous study reported that activation of Rbp4+ L5 pyramidal cells depolarized L2/3 neurons and triggered transitions to the global up state in anesthetized mice. This work proposed that L5-mediated recurrent circuitry enhances and prolongs sustained activity within the cortical column, which is contrary to our data. Although the most obvious difference is their use of anesthesia, we found that the same anesthesia condition (2 mg/kg urethane) did not affect our results and confirmed brain state-independent suppression of superficial layers by L5 activation. One explanation is that, since the majority of their data are LFP or multi-unit recordings with electrodes placed 450–700 μm deep, they may reflect the measurement of optogenetically modulated L5 activity rather than global recurrent activity (see Fig. 1b for distribution of Rbp4+ neurons across A1 cortical depth). Alternatively, since the previous work was conducted in unspecified neocortical areas, potential differences in the circuit wiring between cortical areas could account for the discrepancy. Indeed, previous studies also found opposing roles for L6 corticothalamic neurons between A1 and V1; optogenetic activation of Ntsr1 + L6 neurons suppressed the activity of superficial layers in V1 but enhanced it, whereas the same manipulation in A1 enhanced it. Altogether, these works may indicate that distinct layers across cortical areas provide negative feedback onto superficial layers: L5 in A1 and L6 in V1. Regardless of whether this specific scheme is correct or not, the similar negative feedback circuits found across sensory modalities highlight the importance of feedback suppression from output layers in the cortex.

L5 is known as a layer with the highest spontaneous activity. This high-level basal firing indicates that L5 constantly sparsens and sharpens the activity of superficial layers even in quiescence, which is supported by our optogenetic inactivation data. The extensively associative network between L2/3 neurons sparsens their activity, which may improve the information storage capacity of the cortical circuit. It is noteworthy that, unlike the L5 activation that suppresses L2/3 via both intracortical and subcortical projections, L5 inactivation effects are found to be mediated exclusively by the intracortical pathway. A similar asymmetry between activation and inactivation was also reported in the manipulation of cortical projections onto the inferior colliculus. Thus, the local intracortical loop likely allows for continuous feedback suppression in the basal state, while the more energy-consuming subcortical feedback loop takes effect only during raised activity to ensure sufficient sharpening of cortical activity. In addition, since we used pure tones with relatively high intensity (70 dB SPL) throughout our experiments, L5 recurrent connections may show different contributions to the processing of near-threshold sound stimuli. In the future, it would be interesting to investigate how the recruitment of intracortical and subcortical feedback depends on sound stimulus features, such as the intensity and spectro-temporal structures.

Although the dominant effect of L5 activation is to suppress superficial layers, we also observed transient activation in a small fraction of L2/3 RS neurons. Therefore, our results do not rule out the possibility that phasic activation of L5 neurons may regulate sensory tuning of a sparse population of L2/3 neurons by both excitatory and inhibitory mechanisms. Interestingly, ascending projections from L5 neurons are reported to lack a preference for functionally connected co-tuned subnetworks, which contrasts with L4 → L2/3 or L2/3 → L5 projections. Thus, future work to identify the rules governing L5 → L2/3 connectivity will be critical in understanding how both excitatory and inhibitory feedbacks contribute to cortical sensory processing.

L5 neurons are known to receive most of their excitatory inputs from L2/3 and this descending excitatory drive has been experimentally supported both in vitro and in vivo. These observations place the L5 → L2/3 translaminar inhibitory circuit well poised to function as a negative feedback mechanism that sparsens and stabilizes cortical activity. In contrast, recent studies reported that deep cortical layers also receive direct thalamocortical inputs and are able to maintain their activity in the absence of descending inputs from L2/3 neurons. This finding raises the possibility that L5 could regulate superficial layers independently from its inputs from L2/3, potentially expanding the computational capacity of this translaminar circuit. Convergence of ascending, descending, and lateral inputs onto L5 may allow flexible modulation of cortical sensory processing based on various factors, such as sensory context, attention, and experience.
Methods

Animals. Mice were at least 6 weeks old at the time of experiments. Tg(Rbp4-Cre) KL100Gsat/Mmucd (Rbp4-Cre; MMRRC 037128-UCD) and Tg(Tlx3-Cre) NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-022-30349-w | www.nature.com/naturecommunications were determined by presenting pure tones at nine frequencies (4 – 64 kHz, log-scaled, 70 DB SPL, 200-ms duration, nine trials at 3–4 s intervals. For testing weak LED intensities (Supplementary Fig. 2), smaller trial numbers (1–3 trials per frequency) were used. Tone stimuli were presented in semi-randomized order, and trials with weak LED light were interspersed with trials from each other. For sharp mapping with intrinsic signal imaging, 3, 10, and 30 kHz pure tones (75 DB SPL, 1-s duration) were presented at a 30-s interval. Pure tone stimuli had 5-ms linear rise-fall at their onsets and offsets. Stimuli were delivered to the ear contralateral to the imaging or recording site. Auditory stimulus delivery was controlled by Bpod (Savworks) running on Matlab.

Intrinsic signal imaging. Intrinsic signal images were acquired using a custom tandem lens microscope (composed of Nikkor 35 mm 1:1.4 and 135 mm 1:2.8 lenses) and a 12-bit CMOS camera (DS-1A-01M30, Dalsa) placed in a sound isolation chamber (Gretchen Industries). All mice were first implanted with a custom stainless-steel head-bar. Mice were anesthetized with isoflurane (0.8–2%) vaporized in oxygen (1 L/min), and kept on a feedback-controlled heating pad at 37 °C. Up and down states in A1 spikes were automatically detected and the state, and a 64-channel silicon probe (ASSY-77-H3, sharpened, Cambridge Neurotech) was slowly (approximately 1 μm per second) inserted perpendicular to the brain surface. Spikes were monitored during probe insertion, and the probe was advanced until its tip reached the white matter, where no spikes were observed. The electric current was turned off. In some mice, A1 recordings were conducted in both awake and anaesthetized states successively. After recording in the awake state, mice were deeply anesthetized with urethane (1.5 mg/kg body weight) prior to imaging. Images were acquired with a confocal microscope (Olympus) across the cortical column, images were Z-projected with the maximum intensity, averaged across the tangential axis, and normalized by the sum of intensity across depths. The cortical depth was standardized with 0 for the brain surface and 1 for the pial surface. Brains used for recordings were fixed in 4% paraformaldehyde in the same way, except they did not go through perfusion. Linear probes were inserted into both sides of the brain. Both male and female animals were used and housed at 21 °C and 40% humidity in a reverse light cycle (12 h:12 h). All procedures were approved and conducted in accordance with the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill, as well as guidelines of the National Institute of Health.

Viruses. AAV8.2.hEFla.DIO.synaptophysin.EYFP (AAV-RN2; 2.5 x 1012 genome copies (GC)/mL) and AAV8.2.hEFla.DIO.synaptophysin.mCherry (AAV-RN1; 2.5 x 1012 GC/mL) were obtained from Gene Delivery Technology Core, Massachusetts General Hospital. AAV9.hSynX.72ChromosomeRdTomoato (AV6556C; 2.5 x 1012 GC/mL) was obtained from the University of North Carolina Vector Core. AAV9.hEFla.DIO.eNhPr3.0.EYFP.WRE.hGH (2.89x106, 2.2 x 1013 GC/mL) was obtained from Addgene. CAV-2.2Cre (4.0 x 1013 GC/mL) was obtained from Plateforme de Virologie de Montpellier.

Sound stimulus. Auditory stimuli were calculated in Matlab (Mathworks) at a sample rate of 192 kHz and delivered via a free-field electrostatic speaker (ESI; Tucker-Davis Technologies). Speakers were calibrated over a range of 2–64 kHz to give a flat response (±1 dB). Click sounds were generated as 0.1 ms monopolar rectangular pulse and presented 200 trials at a 30-s interval. Clicks were determined by presenting pure tones at nine frequencies (4–64 kHz, log-scaled, 70 DB SPL, 200-ms duration, nine trials at 3–4 s intervals. For testing weak LED intensities (Supplementary Fig. 2), smaller trial numbers (1–3 trials per frequency) were used. Tone stimuli were presented in semi-randomized order, and trials with weak LED light were interspersed with trials from each other. For sharp mapping with intrinsic signal imaging, 3, 10, and 30 kHz pure tones (75 DB SPL, 1-s duration) were presented at a 30-s interval. Pure tone stimuli had 5-ms linear rise-fall at their onsets and offsets. Stimuli were delivered to the ear contralateral to the imaging or recording site. Auditory stimulus delivery was controlled by Bpod (Savworks) running on Matlab.

Histology. To quantify soma distribution for pan-L5 and IT pyramidal cells, Bp4-Cre×A9, Bp4-Cre×CBA, or Tlx3-Cre mice were injected with AAV9.EF1a.DIO.eNhPr3.0.EYFP.WRE.hGH (280 nL) into the right A1. In the same mice, AAV9.EF1a.DIO.eNhPr3.0.EYFP.WRE.hGH (280 nL) was injected into the right auditory cortex of newborn mice postnatal day 1 (Bp4-Cre×A9, Bp4-Cre×CBA, or Tlx3-Cre) under hypothermia anesthesia, and recordings were conducted after the mice reached six weeks old. For PT neuron inactivation, AAV9.EF1a.DIO.eNhPr3.0.EYFP.WRE.hGH (280 nL) was injected into the right auditory cortex of newborn C57BL/6J or A9 mice (postnatal day 1–3). After the mice reached six weeks old, CAV-2.2Cre (100 nL/site) was injected into three sites within the external cortical shell of the right inferior colliculus, and recordings were conducted four weeks after virus injections. In all experiments, black cement was used during head-cap implantation, and silicone was placed over the exposed skull to prevent light exposure to the auditory cortex between virus injection and recording.

On the day of recording, following a small craniotomy and durotomy in the right A1 identified by intrinsic signal imaging, mice were head-fixed in the awake state and a 64-channel silicon probe (AIPP-77-H3, sharpened, Cambridge Neurotech) was slowly (approximately 1 μm per second) inserted perpendicularly to the brain surface. Spikes were monitored during probe insertion, and the probe was advanced until its tip reached the white matter, where no spikes were observed. The reference electrode was placed at the dura above the visual cortex. The probe was advanced, and before collecting data, 0.3 ms monopolar rectangular pulses were administered, digitized (RHD2164, Intan Technologies), and acquired at 20 kHz with OpenEphys system (https://open-ephys.org). A fiber-coupled LED (Chromireg: 625 nm; eNhPr3.0: 595 nm) was positioned 1.2 mm above the thinned skull and a small aNpyr in the core. In stereo-trials, LED illumination was delivered that lasted 1 s before the onset of the onset of the inferior colliculus, which was not illuminated, and the data from the blank trials were averaged for each stimulus at the onset (0.3 ms) was used to minimize activation of fibers of passage7, except for Fig. 3h and Supplementary Fig. 2a, b. For photoinactivation with eNhPr3.0, constant illumination without ramp was used in Bp4-Cre mice. In Tlx3-Cre and IT multiunits showed photoactivation in Chromireg experiments, and 39.0 ± 3.9% showed photoinactivation in eNhPr3 experiments. During recording, mice sat quietly (with occasional bouts of whisking and grooming) in a loosely fitted plastic tube with a custom-built sound-attenuating enclosure. The tube was lined with foam to further increase the comfort and was further covered with a plastic bag. Ambient light was present in the recording chamber to prevent startling the mice when the LED was turned on. In some mice, A1 recordings were conducted in both awake and anesthetized states successively. After recording in the awake state, mice were injected with urethane (1.5–2.0 g/kg body weight) subcutaneously and kept on a feedback-controlled heating pad at 37 °C. Up and down states in A1 spikes were observed 5–10 min after injection, indicating deep anesthesia. Data collection was started at least 30 min after the urethane injection. In other mice, recordings were conducted successively in A1 and MVG. By inserting a linear probe deeper after A1 recording, we were able to reach MVG, where we observed time-locked click-sound interactions. A1 data for after injection was collected. The recording locations in MVG were confirmed by both short-latency click responses and post hoc identification of probe tracks in the brain sections counterstained with DAPI. In inferior colliculus recording experiments, a head cap was implanted the day before recording without covering the right auditory cortex and the inferior colliculus. Ambient light was visible through the skull, and both the auditory cortex and inferior colliculus were covered with silicone. On the day of recording, a small craniotomy and durotomy were made in

Unit recording with optogenetic manipulation. For pan-L5 and IT neuron activation, Bp4-Cre×A9, Bp4-Cre×CBA, or Tlx3-Cre mice were injected with AAV9.EF1a.DIO.eNhPr3.0.EYFP.WRE.hGH (400–600 nL) into the pial surface, 120 nL/site) at two locations within the right A1, guided by intrinsic signal imaging. For PT neuron manipulations, we chose retrograde viral strategy over transgenic mice since we observed almost no recombination in A1 when we used Tg(Sim1-Cre)K18g sat mice, which were reported to express Cre selectively in L5 PT neurons of the motor cortex14, C57BL/6J or A9 mice were injected with CAV-2.2Cre (100 nL/site) into three sites within the external cortical shell of the right inferior colliculus, which was visualized through the thinned skull (one medial site, 400 μm deep from the pial surface, and two lateral sites, 350 and 920 μm deep). In the same mice, AAV9.hSyn.X.72ChromosomeRdTomoato was injected into L5 at two locations within the right A1. Recordings were conducted two weeks (pan-L5 and IT) or four weeks (PT) after virus injections. It was reported that CAV-2.2Cre injection in the inferior colliculus labels A1 L5 PT neurons as well as a sparse population of L6 neurons close to the white matter15. We avoided labeling deep L6 neurons by injecting Cre-dependent AAVs at a shallower depth (400 μm) in A1 and histologically confirmed the lack of L6 labeling. Surfaces were visualized with a confocal microscope (Nikon Eclipse E800). Histology figure panels were generated by overlaying signals from multiple colors using Fiji software (https://image.net/Fiji).
the inferior colliculus, and a 64-channel silicon probe was inserted down to around 1 mm from the surface. The channels corresponding to the external cortex and central nucleus of the inferior colliculus were maximized by better stimulating for this study because there were no animal treatment groups. All statistical methods but were based on those commonly used in the field.

### Data availability

Source data for all figures are provided with this paper as a supplementary data file. Other data that support the findings of this study are available from the corresponding authors upon reasonable request.

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### References

1. Douglas, J. & Martin, K., Neuronal circuits of the neocortex. Annu. Rev. Neurosci. 27, 419–451 (2004).

2. Binzegger, T., Douglas, R. J. & Martin, K. A. C. A Quantitative map of the circuit of cat primary visual cortex. J. Neurosci. 24, (2004).

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### Statistical analysis

All data are presented as mean ± SEM or median with 25th and 75th percentiles, as stated in the figure legend. Statistically significant differences between conditions were determined using standard two-sided parametric or nonparametric tests in Matlab. Wilcoxon signed-rank test was used for one-sample nonparametric tests. Paired t-test was used for paired data, and Wilcoxon rank-sum test was used for independent group comparisons. Bonferroni correction was used for multiple comparisons, and corrected p values were reported. In cases where parametric statistics are reported, the normality of data distribution was tested with one-sample Kolmogorov-Smirnov test. Randomization is not relevant for this study because there were no animal treatment groups. All “n” values refer to the number of single-units except when explicitly stated that the n is referring to the number of mice, number of unit-frequency pairs, or number of sections.

### Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.
3. Douglas, R. J., Koch, C., Mahowald, M., Martin, K. A. C. & Suarez, H. H. Recurrent excitation in neocortical circuits. Sci. (80-.). 269, 981–984 (1995).

4. Osten, H., Finn, E., Miller, K. D. & Deisseroth, K. Inhibitory stabilization of the cortical network underlies visual surround suppression. Neuron 62, 578–592 (2009).

5. Rubin, D. B., Van Hoesen, S. D. & Miller, K. D. The stabilized supralinear network: a unifying circuit motif underlying multi-input integration in sensory processing. Neuron 85, 402–417 (2015).

6. Kato, H. K., Asinof, S. K. & Isaacson, J. N. Network-level control of frequency tuning in auditory cortex. Neuron 95, 412–423.e4 (2017).

7. Adesnik, H. Synaptic mechanisms of feature coding in the visual cortex of awake mice. Neuron 95, 1147–1159.e4 (2020).

8. Litwin-Kumar, A., Rosenbaum, R. & Dror, B. Inhibitory stabilization and visual coding in cortical circuits with multiple interneuron subtypes. J. Neurophysiol. 115, 1399–1409 (2016).

9. Aponte, D. A. et al. Recurrent network dynamics shape direction selectivity in primary auditory cortex. Neuron 87, 179–192 (2015).

10. McGinley, M. J. J., Davis, S. V. Y. & McCormick, D. A. Cortical membrane potential signature of optimal states for sensory signal detection. Neuron 87, 159–170 (2012).

11. Li, L., Li, Y., Zhou, M., Tao, H. W. & Zhang, L. I. Intracortical multiplication of thalamocortical signals in mouse auditory cortex. Neuron 87, 1143–1161 (2015).

12. Seybold, B. A., Phillips, E. A. K., Schreiner, C. E. & Hasenstaub, A. R. Inhibition of visual cortical layers with network integration. Neuron 87, 1181–1192 (2015).

13. Ayaz, A. & Chance, F. S. Gain modulation of neuronal responses by subtractive and divisive mechanisms of inhibition. J. Neurophysiol. 101, 958–968 (2009).

14. Atallah, B. V., Bruns, W., Carandini, M. & Scanziani, M. Parvalbumin-expressing interneurons linearly transform cortical responses to visual stimuli. Neuron 73, 159–170 (2012).

15. Osten, S., Bortone, D., Adesnik, H. & Scanziani, M. Gain control by layer six in cortical circuits of vision. Nature 483, 47–52 (2012).

16. Osten, S. et al. Maintenance of persistent activity in a frontal thalamocortical network. Neuron 84, 545, 1787–1797 (2015).

17. Vecchia, D. et al. Temporal sharpening of sensory responses by layer V in the mouse primary somatosensory cortex. Curr. Biol. 30, 1589–1599.e10 (2020).

18. Blackwell, J. M., Lesicko, A. M., Rao, W., De Biasi, M. & Geffen, M. N. Auditory cortex shapes sound responses in the inferior colliculus. Elife 9, e51890 (2020).

19. Tomm, C., Sarria, F. & Petersen, C. The excitatory neuronal population of the C2 barrel column in mouse primary somatosensory cortex. Neuron 30, 826–836 (2010).

20. Zaman, M., Willson, R., Hancox, K. M. & Polley, D. Sensory overamplification in layer 5 auditory corticofugal projection neurons following cochlear nerve synaptic damage. Nat. Commun. 9, 1192 (2018).

21. Osten, S. et al. Laminar and columnar organization of ascending excitatory projections to layer 2/3 pyramidal neurons in rat barrel cortex. J. Neurosci. 26, 5670–5679 (2005).

22. Hooks, B. M. et al. Laminar analysis of excitatory local circuits in vibrissal motor and sensory cortical areas. PLoS Biol. 9, 1000572 (2011).

23. Shepherd, G. M. G. & Svoboda, K. Laminar and columnar organization of ascending excitatory projections to layer 2/3 pyramidal neurons in rat barrel cortex. J. Neurosci. 25, 5670–5679 (2005).

24. Hooks, B. et al. Organization of corticofugal and thalamic output to pyramidial neurons in mouse motor cortex. J. Neurosci. 33, 748–760 (2013).

25. Beltramo, R. et al. Layer-specific excitatory circuits differentially control recurrent network dynamics in the neocortex. Nat. Neurosci. 16, 227–234 (2013).

26. Marschel, J. H. et al. Cortical layer-specific critical dynamics triggering transition. Sci. (80-.). 339, 865–872 (2012).

27. Jiang, X. et al. Principles of connectivity among morphologically defined cell types in adult neocortex. Sci. (80-.). 350, 1a9462 (2015).

28. Klapoetke, N. C. et al. Independent optical excitation of distinct neural populations. Nat. Methods 11, 338–346 (2014).

29. Gerfen, C., Paletzki, R. & Henst, N. GENSAT BAC Cre-recombinase driver lines to study the functional organization of cerebral cortical and basal ganglia circuits. Neuron 80, 1368–1383 (2013).

30. Tasic, B. et al. Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. Nat. Neurosci. 19, 335–346 (2016).

31. Vecchia, D. et al. Spatial sharpening of sensory responses by layer V in the mouse primary somatosensory cortex. Curr. Biol. 30, 1589–1599.e10 (2020).

32. Asokan, M., Williamson, R., Hancox, K. M. & Polley, D. Sensory overamplification in layer 5 auditory corticofugal projection neurons following cochlear nerve synaptic damage. Nat. Commun. 9, 1192 (2018).

33. Peters, A. & Payne, B. R. Numerical relationships between geniculocortical afferents and pyramidal cell modules in cat primary visual cortex. Cereb. Cortex 3, 69–78 (1993).

34. Sanzeni, A. et al. Inhibition stabilization is a widespread property of cortical networks. Elife 9, e54875 (2020).

35. Bortone, D., Osten, S. & Scanziani, M. Translaminar inhibitory cells recruited by layer 6 corticocortical neurons suppress cortical circuitry. Neuron 82, 474–485 (2014).

36. Kavarday, Z. F., Martin, K. A. C., Somogyi, P. & Friellander, M. J. Evidence for interlaminar inhibitory circuits in the striate cortex of the cat. J. Comp. Neurophysiol. 60, 19–197 (1987).

37. Kapfer, C., Glickfeld, L. L., Atallah, B. V. & Scanziani, M. Supralinear increase of recurrent inhibition during sparse activity in the somatosensory cortex. Nat. Neurosci. 20, 743–753 (2017).

38. Naka, A. et al. Complementary networks of cortical somatostatin interneurons enforce layer specific control. Elife 8, e43696 (2019).

39. Negro, M. J., Hashikawa-Yamasaki, Y. & Rudy, B. Diversity and connectivity of layer 5 somatostatin-expressing interneurons in the mouse barrel cortex. J. Comp. Neurophysiol. 38, 1622–1633 (2018).

40. Klinke, A. M. et al. Inhibitory gating of coincidence-dependent sensory binding in secondary auditory cortex. Neuron 12, 4610 (2021).

41. Kline, A. M. et al. Complementary control of sensory adaptation by two types of cortical interneurons. Elife 4, e5415 (2015).

42. Natana, R. et al. Sensory responses in the neocortex driven by behavioral relevance. Neuron 88, 1027–1039 (2015).

43. Lakunina, A. A., Nardoci, M. B., Ahmadina, Y. & Jaramillo, S. Somatostatin-expressing interneurons in the auditory cortex mediate sustained suppression by spectral surround. J. Neurosci. 40, 3584–3575 (2020).

44. Reyes, A. & Sakmann, B. Developmental switch in the short-term modification of unitary EPSPs evoked in layer 2/3 and layer 5 pyramidal neurons of rat neocortex. J. Neurosci. 19, 3827–3835 (1999).
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Author contributions

K.O. conducted all the experiments. K.O. and H.K.K. designed the project, analyzed data, and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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