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Review

Layer 1 neocortex: Gating and integrating multidimensional signals

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SUMMARY

Layer 1 (L1) of the neocortex acts as a nexus for the collection and processing of widespread information. By integrating ascending inputs with extensive top-down activity, this layer likely provides critical information regulating how the perception of sensory inputs is reconciled with expectation. This is accomplished by sorting, directing, and integrating the complex network of excitatory inputs that converge onto L1. These signals are combined with neuromodulatory afferents and gated by the wealth of inhibitory interneurons that either are embedded within L1 or send axons from other cortical layers. Together, these interactions dynamically calibrate information flow throughout the neocortex. This review will primarily focus on L1 within the primary sensory cortex and will use these insights to understand L1 in other cortical areas.

INTRODUCTION

Our capacity to seamlessly reconcile real-time sensory information allows us to navigate a rapidly changing world. Although all six layers of the neocortex contribute to the processing of sensory information, the dense top-down projections present in layer 1 (L1) make it one of the key sites where these input signals are trafficked (Figure 1A). By being a central terminal that harbors higher-order information, L1 is likely central to the evolution of higher cortical function. This is evident by the disproportionally massive expansion in the size of supragranular layers in primates, especially L1.[1,2] This expansion is accompanied by the increased complexity of wiring and the emergence of specialized features in conserved cell types in L1 of human neocortex, such as GABAergic rosehip neurons and interlaminar astrocytes.[3–6] These evolutionary changes may reflect the importance of L1 in processing complex information that is required for higher cognition. Despite the differences between rodents and humans, the fundamental organization of L1 is conserved. However, the wealth of inputs contributing to L1 function and complexity of their interactions posed a challenge to its systematic study. As such, to date, our understanding of L1 has been limited due to the broad combination of methods required to study it. Only recently, with the advent of viral and genetic techniques, combined with optogenetic and imaging approaches, have we been able to achieve the resolution necessary to comprehend the circuit organization of L1 and begin to speculate about its underlying functional role.

L1 is distinct from other cortical layers in that it is largely devoid of excitatory cell bodies. Instead, it is primarily composed of the apical dendrites of excitatory pyramidal neurons, whose cell bodies reside outside of L1, complemented by a population of inhibitory neurons known as L1 interneurons and the dendrites of other inhibitory interneurons (Figure 1B). This unique anatomical organization positions L1 as a crucial hub for integrating information. Inputs to L1 predominantly synapse onto the apical dendritic tufts of excitatory pyramidal neurons. These include excitatory "top-down" inputs from other cortical and thalamic regions involved in higher-level cognitive processes, complemented by excitatory local inputs from L5 intratelencephalic (IT) pyramidal neurons and L6b subplate neurons. This information is modulated by various neuromodulatory inputs, such as cholinergic and noradrenergic, and is gated by local inhibitory inputs from L1 interneurons and Martinotti-type somatostatin-expressing (SST+) interneurons located outside of L1.[7–10] The joint incorporation of excitatory, modulatory, and inhibitory inputs to L1 allows sophisticated information processing in apical dendritic tufts of pyramidal neurons, complemented by the "bottom-up" sensory inputs that primarily target their cell bodies in other layers (Figure 1A). This organization enables the proper integration of sensory information with internal signals across different compartments of pyramidal neurons and offers flexibility in the interpretation of sensory information under varying behavioral contexts.

In this review, our goal was to summarize the latest findings on neocortical L1 and generalize common principles that can help facilitate future research. Although we will touch upon its role in different cortical regions, our main discussion will center around the primary sensory cortex in mice, where we have a better understanding of the anatomy and function of different inputs. We will start by providing an overview of the excitatory and neuromodulatory inputs in L1, surveying their anatomical

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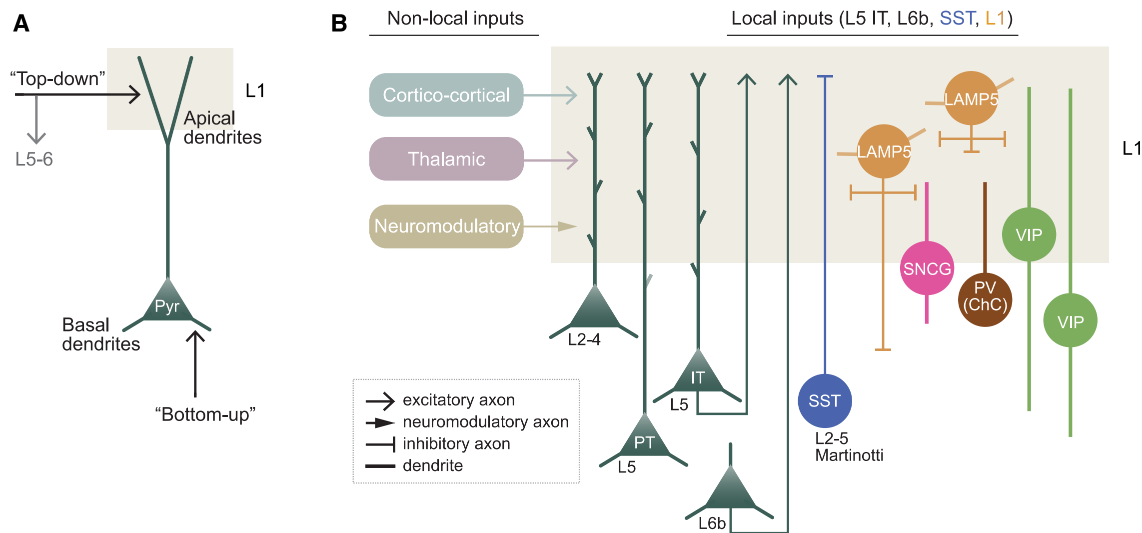

Figure 1. Illustration of the components present in L1

(A) Cortical pyramidal neurons possess both basal and apical dendrites. Most pyramidal neurons (except L6) have apical dendrites that bifurcate and extend apically into L1. "Top-down" signals, including higher-order cortical and thalamic regions, primarily reach L1 and infragranular layers (L5 and L6) in the sensory cortices. Top-down projections to L1 target the apical dendritic tufts in L1, whereas "bottom-up" ascending inputs primarily target the somatic regions and basal dendrites in other layers outside of L1. Pyr, pyramidal neuron.

(B) In the sensory cortex of mice, L1 is comprised of the following elements: inputs such as (1) non-local excitatory inputs, including cortico-cortical and thalamic inputs, (2) excitatory inputs originating from local L5 IT and L6b subplate neurons, (3) inhibitory inputs from Martinotti-type SST+ interneurons, and (4) neuromodulatory inputs; dendrites from excitatory pyramidal neurons in L2-5, as well as various inhibitory interneurons such as vasoactive intestinal peptide-expressing (VIP+), parvalbumin-expressing (PV+, including the chandelier cells [ChC]), and synuclein gamma-expressing (SNCG+) interneurons; L1 interneurons, including lysosomal associated membrane protein family member 5-expressing (LAMP5+); and VIP+ interneurons, whose cell bodies are in L1. The majority of LAMP5+ interneurons (mostly the NDNF+ population) have their axons restricted within L1, whereas the LAMP5+ interneurons that do not express NDNF (the NDNF− population; single-bouquet) can have their axons extending to deeper layers.

organization and the signals they convey. Having outlined the inputs, we will explore how these inputs are directed, sorted, and integrated into the apical dendritic tufts of pyramidal neurons. We will then discuss the different inhibitory inputs to L1 and how they gate L1 activity. Finally, we will delve into the non-neuronal structures that are proximate to L1, such as pia vessels and meninges, and explore how they may contribute to L1 function and affect L1 in pathological conditions. Through this review, we aim to unravel the complexity of this crucial cortical layer, whose function[11–15] and development (which have been extensively reviewed elsewhere)[16–19] have recently garnered much interest.

THE SOURCE AND FUNCTION OF EXCITATORY INPUTS TO L1

Since L1 serves as a confluence for receiving and integrating various information, it is important to review the individual excitatory inputs present in L1 and the information they carry. To do so, we focus on the primary sensory cortex, particularly the primary visual cortex (V1) in mice, as a representative example and examine its inputs systematically (Figure 2A). First, we will explore the thalamic inputs, including both the higher-order and first-order thalamic inputs. Subsequently, we will delve into the cortico-cortical feedback inputs by examining inputs from hierarchically higher visual cortical areas, as well as inputs from higher association cortical areas. Finally, we will investigate other excitatory inputs, such as inputs from other layers within V1, contralateral inputs, cross-modal inputs, and inputs from orbital and temporal cortical areas.

Thalamic inputs

The higher-order thalamus densely targets L1 and is the major thalamic input to L1 of V1. It includes inputs from the lateral posterior (LP), which, together with the lateral dorsal (LD) nuclei, comprise the higher-order visual thalamic nuclei.[32] LP inputs have larger receptive fields and less confined retinotopic organization compared with the first-order dorsal lateral geniculate nucleus (dLGN) inputs.[21] These higher-order inputs have been suggested to encode discrepancies between self-generated motion and visual flow and convey this information to V1.[21,33,34] LP inputs also perform a "noise-canceling" sharpening effect on visual responses of L2/3 pyramidal neurons in V1 by recruiting L1 inhibition, contributing to visual discrimination in mice.[35]

The first-order thalamic input to V1 comes from dLGN, which is the primary visual thalamic nucleus. dLGN axons target L4 densely in V1 and extend to L1 and L5b/6. These axons, primarily relaying peripheral visual information to V1, exhibit small receptive fields and a high degree of retinotopic organization.[21] Interestingly, the subset of dLGN neurons that project to L1 of V1 may not be purely first order. dLGN is subdivided into the following two parts: the core and the shell. Retrograde tracing studies indicate that the projections to L1 originate from the shell region of dLGN.[20,22,23,36,37] Although both core and shell receive inputs from the retina, the shell also receives inputs from the retinal direction-selective ganglion cells and superior colliculus

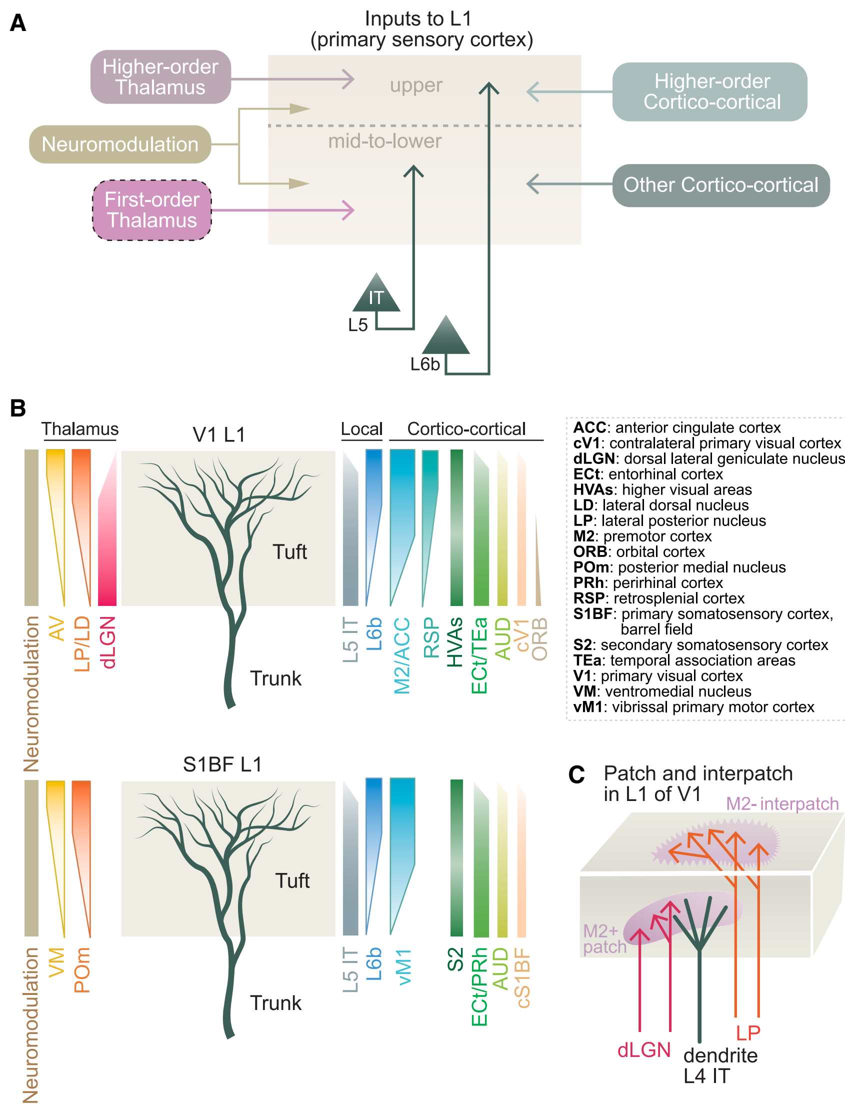

Figure 2. Intralaminar targeting preferences of different inputs in L1 of V1 and S1BF

(A) Inputs to L1 exhibit specificity in intralaminar targeting in mice. Higher-order cortico-cortical and thalamic inputs, along with local L6b subplate inputs, show a preference for targeting upper L1 in primary sensory cortices. In contrast, first-order thalamic inputs, local L5 IT neurons, and inputs from other cortical regions prefer mid-to-lower L1. Dashed border around first-order thalamus indicates that the projection from these populations to L1 is complicated (also see main text). First, it is not clear whether it projects to L1 in some cortical regions (e.g., VPM to S1BF in mice). Second, the subregion in the first-order thalamus that projects to L1 may not be purely "primary" (e.g., the shell region of dLGN).[20] Neuromodulatory inputs have a broader projection pattern across L1.

(B) Intralaminar preferences of various inputs to L1 of V1 (upper panel) and S1BF (lower panel) in mice. Refer to Table 1 for complete anatomy abbreviations. Each input is labeled with a different color, and the color gradient indicates the density of each input across L1. For regions where the inputs preferentially target the upper portion of L1 in V1, the boundaries are labeled with colored lines. These results are summarized from previous studies[7,8,13,21–29] and Allen Mouse Brain Connectivity Atlas: connectivity.brain-map.org/projection. Dark green branches represent the dendrites of pyramidal neurons, where their tufts are mostly located in L1. The intralaminar targeting pattern may also allow different inputs to integrate at distinct sub-compartments within the tufts. Note that in V1, the preference of HVA inputs for specific portions within L1 of V1 can vary depending on the specific input areas (refer to the main text for details).[30] In S1BF, there are variabilities among studies for the intralaminar targeting pattern of S2 input to S1BF.[13,26,31]

(C) The M2-AChR-expressing patches and M2-AChR-lacking interpatches in L1 of V1 in mice. They are spatially non-overlapping structures in L1 as viewed from the top in a flat mount preparation. LP inputs predominantly terminate in M2-AChR-lacking interpatches, whereas dLGN inputs overlap with M2-AChR-expressing patches. M2-AChR-expressing patch is labeled as "M2+ patch" and M2-AChR-lacking interpatch is labeled as "M2− interpatch."

involved in visual motion processing.[38] The dLGN neurons projecting to L1 also have collaterals in L4, raising the possibility that these L1/L4 targeting shell neurons carry both visual and directional information.[39–41] Similarly, inputs from the ventral medial geniculate body (MGBv, first-order auditory thalamic nucleus) have been found in L1 of the primary auditory cortex (A1), and L1 neurons in A1 possess auditory-evoked responses.[42,43] Notably, sensory-evoked responses have been recorded in response to whisker stimulation in L1 neurons of the barrel cortex of mice.[44–47] However, it has been argued whether somatosensory information carried via the ventral posteromedial nucleus (VPM) projects directly to L1 of the primary somatosensory cortex (S1).[7,8,48,49]

Cortico-cortical feedback inputs

Cortico-cortical feedback projections densely target L1, playing a crucial role in higher-order cognitive processes such as attention, learning, and prediction. These inputs to V1 can be classified into the following two categories: (1) inputs from higher visual areas and (2) inputs from higher association areas, including the retrosplenial (RSP) and the premotor or anterior cingulate cortex (M2/ACC).

Higher visual areas receive feedforward information from V1 and reciprocally provide feedback inputs to V1 that synapse on multiple layers, including L1. The distribution of these projections varies based on the source. Feedback inputs from higher visual areas to V1 are spatially organized, enhancing visual responses and contributing to the context-dependent modulation of visual processing.[50] One example of such modulation is surround modulation, where neurons adjust their response to local visual features within their receptive fields depending on the visual context simultaneously presented in the surrounding receptive region.[51] This ability of neurons to adapt their responses based on the surrounding scene helps with visual prediction.[52–54] Interestingly, this feature is partially generated through the local arrangement of inputs from higher visual areas, such as

lateromedial area (LM, analogous to V2 in primates), with spatially offset receptive fields converging on a given retinotopic site in V1.[55,56] Boutons in L1 from these inputs are likely involved in this function.[51,55]

The other types of cortico-cortical feedback inputs are from RSP and M2/ACC, which target L1, L5, and L6 in V1.

RSP, a multisensory area that encodes visual, vestibular, and motor-related information, is crucial for spatial navigation and other higher cognitive functions.[57,58] Its inputs convey head-motion signals to L5 and L6 of V1; however, it remains unclear whether this information is also sent to L1.[59,60] RSP boutons in L1 of V1 exhibit increased activity and a ramping-up response during visually guided active avoidance tasks, suggesting a potential role in associative learning in V1.[61]

M2/ACC, considered a homolog of the primate medial eye field, also plays an important role in modulating sensory processing.[62] Focal activation of M2/ACC axons in L1 of V1 increases the firing of nearby V1 neurons at their preferred orientations while reducing the activity of neurons located several hundred micrometers away. This suggests that M2/ACC input can modulate gain in visual response and participate in surround modulation by engaging local inhibitory circuits.[63] Additionally, M2/ACC input to V1 is selectively activated after error trials in attention tasks and contributes to post-error performance adjustment, indicating its active role in conveying attention signals.[64] M2/ACC input also contributes to visual-motor integration. Its axons in L1 of V1 transmit motor-related signals preceding locomotion onset, and its activation induces turning behavior in rodents, possibly due to the perception of illusory visual flow.[65,66] Taken together, these findings demonstrate that M2/ACC inputs in L1 of V1 play a crucial role in attention, contextual modulation of visual processing, and visual-motor integration.

Additional cortical excitatory inputs

Apart from thalamic inputs and cortico-cortical feedback inputs, L1 of V1 receives inputs from infragranular layers within V1, the auditory cortex, the contralateral V1 (cV1), the orbital cortex (ORB), as well as the temporal cortical areas.[7]

Excitatory neurons within V1, such as L5 IT and L6b subplate neurons send translaminar projections to L1. The specific functional distinctions between L5 IT and L6b inputs to L1 are not fully understood. However, they are both driven by higher-level inputs, such as top-down inputs and L2/3 IT neurons, and may contribute to regulating feedback signals that are vital for L1 function.[10,67,68] For example, inputs from RSP and M2/ACC, as discussed earlier, target not only L1 but also L5 and L6 in V1. It is tempting to speculate that these projections may carry information transmitted back to L1 through the local excitatory neurons in L5 and L6. Although there is no clear evidence that L2/3 IT neurons send input to L1 in V1,[22,69] L2/3 IT neurons in somatosensory and motor cortices have been shown to have projections in local L1.[22,36,69,70] Further investigation would help determine if L2/3 IT neurons generally contribute input to L1. Furthermore, the local connectivity of excitatory neurons between layers may vary across different cortical regions, resulting in different local projection patterns.[71]

Neuronal responses in primary sensory cortices are also modulated by inputs from other sensory modalities. For example, sound stimulation has an overall inhibitory effect on V1 activity, which is likely mediated via projections from the auditory cortex to L1 of V1 in mice.[72–74] This cross-modality suppression could improve sensory detection thresholds and reaction speeds.[73,75] Notably, sensory stimuli, such as sound, also trigger changes in neuromodulation that affect internal state and behavior in awake subjects.[76] Therefore, one must distinguish the direct effects of other sensory modalities on L1 of V1 versus those mediated by altering internal state and behavior.

Contralateral cortical inputs, also known as callosal projections, connect neurons between the two brain hemispheres. cV1 projects predominantly to L2, L5, and L6 in V1 and is important for binocular visual function.[77] Such inputs appear across cortical regions, reflecting the bilateral organization of the neocortex.[78] Although these cV1 inputs also extend to L1, their specific roles remain unclear. Specifically, L5 IT neurons, one of the local input sources discussed above, also project contralaterally from cV1 to V1 and target multiple layers, including L1.[24]

Finally, there are various less studied cortical regions, such as ORB and the temporal cortical areas, including temporal association areas (TEa), the entorhinal cortex (ECt), and the perirhinal cortex (PRh), all of which have projections to L1 of sensory cortices, including V1, and contribute to sensory associative learning.[25,79,80]

It is important to highlight that many projections targeting L1 also possess collaterals extending into infragranular layers. Although this review primarily focuses on L1, it is crucial to view projections to both L1 and infragranular layers holistically when considering the system's overall functional role. As such, further exploration of these functional interrelationships would indeed be enlightening.

Through this comprehensive description of all inputs to L1 of V1, one can appreciate the vast variety of information converging onto this thinnest layer of cortex. The diverse inputs to L1 highlight its crucial role in signal processing, as it receives and integrates information from multiple sources. The presence of signals independent of the bottom-up afferents in L1 suggests its potential involvement in modulating sensory processing through factors such as context, internal state, and other sensory modalities in sensory cortices. Despite the many types of inputs L1 receives, activation of inputs from even a single source may drive marked changes in neuronal response and even behavioral outcome. How L1 mediates the integration and gating of different information to achieve precise, flexible, effective, and timely modulation of pyramidal neuron activity will be explored in the following sections.

THE SPATIAL ORGANIZATION OF EXCITATORY INPUTS TO L1

The diverse inputs into L1 described above are spatially organized. This precise anatomical arrangement may hold significant implications for our understanding of its function. Within L1, the majority of inputs show preferential innervation, targeting either the upper or mid-to-lower portion (Figure 2A).[7–9] In V1, inputs targeting upper L1 include (1) higher-order thalamic inputs, such as inputs from LP and LD; (2) cortico-cortical feedback inputs originating from specific higher visual areas such as posteromedial (PM) and rostrolateral area (RL), as well as higher association

areas including RSP (more restricted) and M2/ACC (less restricted); and (3) additional cortical excitatory inputs derived from local L6b subplate neurons.[7–9,30] By contrast, inputs innervating mid-to-lower L1 include (1) first-order thalamic input from dLGN; (2) cortico-cortical feedback inputs from certain higher visual areas such as LM and anterolateral area (AL); and (3) additional cortical inputs originating from L5 IT neurons in V1, from cortical regions more relevant to sensory processing, including cV1 and the auditory cortex, and from ORB and the temporal cortical areas, such as TEa and ECt (Figure 2B, upper panel).[7–9,30] As such, inputs from higher-order thalamic nuclei and cortico-cortical feedback pathways predominantly target the upper L1, whereas inputs from regions more directly related to sensory processing, including local L5 IT neurons, ORB, and the temporal cortical areas, are largely restricted to mid-to-lower portions of L1 (Figure 2B, upper panel).

These organizational principles are mirrored in other sensory and motor cortices as well as association areas.[81,82] In S1 barrel field (S1BF), inputs from higher-order thalamic nuclei—such as the ventromedial nucleus (VM) and the posterior medial nucleus (POm), cortico-cortical feedback inputs from vibrissal primary motor cortex (vM1), and additional cortical excitatory inputs from local L6b subplate neurons exhibit a preference for targeting the upper L1.[7–9,30] Inputs from local L5 IT neurons, the auditory cortex, and contralateral S1BF, as well as the temporal cortical areas, which are ECt and PRh, preferentially target the mid-to-lower portion of L1 (Figure 2B, lower panel).[7–9,30] In the prefrontal cortex (PFC), axons from VM predominantly target the upper L1, whereas axons from the thalamic medial dorsal nucleus (MD) and claustrum prefer the mid-to-lower and lower portions of L1, respectively.[83] Therefore, across the neocortex, there exists a dichotomy of higher-order inputs targeting L1 superficially and (relatively) lower-order inputs targeting lower L1.

Complementing the intralaminar organization, inputs to L1 also form distinct spatial domains. In mouse V1, two complementary domains have been identified within L1: muscarinic type 2 acetylcholine (ACh) receptors (M2-AChR)-expressing patches and M2-AChR-lacking interpatches.[30,84,85] The inputs that target specific portions of L1 often show a preference for patch or interpatch regions. For instance, inputs from LP target the upper L1 and M2-AChR-lacking interpatches, whereas inputs from dLGN project to mid-to-lower L1 and overlap with M2-AChR-expressing patches (Figure 2C). Given that M2-AChR functions through inhibitory G protein-coupled receptor (GPCR) signaling, its selective expression may indicate an inhibitory role in gating specific inputs. Although these spatial domains have been studied in mouse V1, it remains uncertain whether similar domains exist in other cortical regions and species.

Complementing these complex but organized excitatory inputs are neuromodulatory and inhibitory signals in L1 that act to modulate and gate excitation, respectively. We next review their organization, and later, we will discuss how they weigh and direct the actions of these excitatory inputs within L1.

NEUROMODULATORY INPUTS TO L1

The neuromodulatory inputs, including cholinergic, noradrenergic, serotonergic, and dopaminergic inputs, play crucial roles in regulating sensory perception, plasticity, and cognitive function. Unlike excitatory inputs, which are region restricted and demonstrate intralaminar preference in L1, neuromodulatory projections are generally widespread and diffuse (Figure 2).

Cholinergic axons originating from the basal forebrain regulate arousal, stimulus salience, learning, and plasticity by releasing ACh in a spatiotemporally precise manner.[42,86–89] Cholinergic neurons projecting to the sensory cortex usually show region- and layer-targeting preferences in mice.[90,91] However, the subset of cholinergic neurons that have axonal arborizations exclusively in L1 project broadly, positioning them to broadcast information throughout the neocortex.[92] Cholinergic activity increases during active behavior states, as indicated by increased locomotion and pupil dilation.[93,94] Specifically, cholinergic axons in L1 show a high correlation with active whisking in S1BF.[95] In L1 of the auditory cortex, they respond to sensory and aversive stimuli, such as auditory tone and foot shock, respectively.[87,96] These results indicate that cholinergic signals in L1 are associated with state changes and aversive stimuli, and these signals are broadly transmitted to L1 across multiple cortical regions. Nevertheless, it remains unclear whether the signals conveyed by cholinergic neurons projecting exclusively to L1 differ from those targeting other layers.

Noradrenergic projections originating from the locus coeruleus also exhibit a broad distribution throughout the neocortex.[97,98] Pupil diameter is a behavior indicator of arousal state, and rapid pupil dilation is associated with phasic activity in noradrenergic axons in L1 of the sensory cortex.[94] These findings suggest that noradrenergic input plays a crucial role in regulating arousal throughout the neocortex, including L1.[99,100]

Serotonergic and dopaminergic inputs have been less studied in the neocortex. Serotonergic input, originating from the dorsal raphe nucleus, is involved in the modulation of behavioral states, sensory perception, plasticity, and reward processing.[101] Its projections and the expression of serotonergic receptors in the neocortex may differ between rodents and primates.[4,102] In mice, serotonergic input shows decreased density along rostro-caudal and ventral-dorsal axes and layer preference for L1, L5a, and deep L6 in the dorsal regions.[103,104] Dopaminergic inputs are sparse in the neocortex and biased toward the rostral regions in mice.[7,13]

In summary, neuromodulatory inputs to L1 are in general diffusive and function to broadcast signals, including behavior states and arousal, across cortical regions. By targeting L1, these inputs can shape L1 activity by modulating the dendritic activity of pyramidal neurons, recruiting cortical inhibitory circuits, or regulating presynaptic excitatory input activity. We will discuss these mechanisms in more detail later.

L1 INHIBITORY INTERNEURONS

With a massive number of different excitatory inputs converging in L1, inhibition in L1 plays a critical role in gating L1 activity. L1 receives inhibitory inputs from two main sources: interneurons within L1 and the axons originating from Martinotti-type SST+ interneurons. We will discuss the inhibition from both interneuron types later in the section gating incoming information: dendritic inhibition in L1. In this section, our focus will be on the

Figure 3. Gene expression in mouse and human L1 of V1

Dotplot showing expression of neurodevelopmental disorders risk genes, neuromodulator receptors, neuropeptides, adhesion molecules, and ion channels within neuronal populations present in L1 of mouse and human V1 using the V1 SMART-Seq v4 RNA sequencing datasets from the Allen Brain Institute.[4,71] The color bar represents the values of gene expression log 10 normalized as counts per million (CPM), and the size of the dot represents the percentage of cells expressing that gene. Genes expressed in less than 5% of the cells and with counts less than 0.6 CPM were filtered out. For this analysis, only inhibitory interneurons from L1 were considered and grouped in three main clusters in mice L1: LAMP5+/NDNF+ (neurogliaform [NGF] and canopy

(legend continued on next page)

interneurons that are resident within L1, where we will discuss their different subtypes, outputs, inputs, and *in vivo* activities.

Subtypes of L1 interneuron

The composition of interneurons within L1 is shared throughout the neocortex. However, the relative proportions of different subtypes may vary. Additionally, the diversity of interneuron subtypes in L1 is greater in the human neocortex, suggesting an increased complexity may be necessary to achieve fine inhibitory regulation in this specific cortical layer in humans.[4] Although there are differences in L1 interneuron subtypes between primates and mice (also see Figure 3 for differences in gene expression between human and mouse L1 interneurons), our focus is specifically on these subtypes in mice.[3–5,105] In the latter, L1 interneurons can be classified into two main groups based on their transcriptional, morphological, and electrophysiological characteristics: lysosomal-associated membrane protein family member 5-expressing (LAMP5+) and vasoactive intestinal peptide-expressing (VIP+) interneurons.[18,24] LAMP5+ interneurons account for ∼90% of L1 interneurons, whereas VIP+ interneurons make up the remaining 10%. LAMP5+ interneurons can themselves be divided based on the expression of the neuron-derived neurotrophic factor (NDNF) gene. NDNF+ interneurons, which constitute ∼80% of LAMP5+ L1 interneurons, typically exhibit elongated axonal arborization restricted within L1. On the other hand, the NDNF− interneurons generally display a single-bouquet morphology and express cholinergic receptor nicotinic α7 subunit (CHRNA7+).[106] Interestingly, the distribution of some interneuron subtypes shows intralaminar preference. CHRNA7+ interneurons typically reside in the lower portion of L1, and VIP+ interneurons are located at the border of L1 and L2. These two subtypes have their axons descending to deeper cortical layers. We will refer to them as NDNF+ (LAMP5+, elongated), CHRNA7+ (LAMP5+, single-bouquet), and VIP+ interneurons in the following discussion.

NDNF+ interneuron-mediated inhibition

NDNF+ interneurons are the majority of L1 interneurons. Approximately half of them are classically defined as neurogliaform (NGF) cells. NGF cells are believed to function via volume transmission. They do not form selective efferent connectivity like other interneurons. Instead, they broadly inhibit neurons (including cell bodies of L1 interneurons as well as dendrites of excitatory and inhibitory neurons) residing in their vicinity. The synapses they form with postsynaptic neurons have larger synaptic clefts and lack classical postsynaptic structures.[107] Their activation induces large and slow-decaying inhibitory potential in postsynaptic neurons, possibly generated by GABA spillover that activates both slow $GABA_A$ and $GABA_B$ receptors at extrasynaptic sites.[108] Therefore, NGF cells provide strong, slow-decaying inhibition in a non-selective manner.

The other half of the NDNF+ population are known as canopy cells, which have their axonal arbors preferring the upper half portion of L1 and show slight differences in efferent properties when compared with NGF cells.[106] Activation of canopy cells induces smaller and slow-decaying (faster than NGF cells but still slow in general) inhibitory postsynaptic potentials, which involve only $GABA_A$ receptors, in a smaller number of postsynaptic neurons.[106] Whether they have selective efferent connectivity and whether the synapses formed by canopy cells are similar to those formed by NGF cells remains unknown.

Nevertheless, both populations of NDNF+ interneurons show a high probability of connecting with each other. This contributes to the mutual inhibition among NDNF+ interneurons, whereby the activation of one NDNF+ interneuron inhibits nearby NDNF+ interneurons, creating a network of reciprocal inhibition *in vivo*.[25,45,69,106,107] This allows for the spatial control of inhibition, resulting in NDNF+ interneurons within specific columns being stimulated, whereas those in adjacent cortical columns are suppressed. The hypothesis that the L1 interneurons in the study are probably NDNF+ interneurons is drawn from the high prevalence of NDNF+ L1 interneurons and their identification by their distinctive elongated morphology.[47]

Diverse afferents to subtypes of L1 interneuron

Different sources of input to L1 can selectively target different subtypes of L1 interneurons. In L1 of V1, inputs from regions such as M2/ACC and RSP specifically target NDNF+ interneurons but avoid CHRNA7+ interneurons.[22] Similarly, in L1 of PFC, VM inputs preferentially target NDNF+ interneurons, whereas MD inputs primarily recruit VIP+ interneurons.[81,109] Even for synapses formed between a single input source and one subtype of L1 interneurons, there are marked differences in the synaptic strength onto individual neurons. In L1 of V1, M2/ACC inputs preferentially target NGF cells; however, the strength of these synapses shows large variability among individual NGF cells.[22]

cells), LAMP5+/NDNF− (Chrna7-expressing single-bouquet cells), and VIP+ cells; four clusters in human L1: LAMP5+/NDNF+ (neurogliaform cells), LAMP5+/NDNF− (putative rosehip cells), LAMP5−/NDNF+/MC4R+ (putative canopy cells), and VIP+ cells.

(A) Risk genes for ASD, schizophrenia, and X-linked intellectual disability. Overall, more risk genes are expressed by human L1 clusters; however, some show higher levels of expression in mouse L1 clusters (e.g., Cul1, Grin2b, Syp, and Gdl1).

(B) Neurotransmitters and neuromodulators receptor genes. Metabotropic glutamate receptor expression is higher in mouse compared with human L1 (e.g., Grm5, Grm7, and Grm8). Cholinergic receptors show different distributions in human and mouse clusters. Chrna7 in humans is expressed mainly by VIP+ cells, while it is mainly expressed in the NDNF− cluster in mouse L1. Furthermore, in humans, we find Chrna4 and Chrna2 expressions in NDNF+ and VIP+ clusters, respectively, whereas in mice, there is almost no expression. Among the neuromodulatory receptors Adra1 is most highly expressed in NDNF+ cluster in human.

(C) Adhesion molecules genes. Cadherins show higher expression in mouse L1. Iglon adhesion molecules are mostly expressed in VIP+ cluster in mouse and in NDNF+ cluster in humans.

(D) Neuropeptides genes. Most of the neuropeptide genes are mainly expressed by VIP+ cluster in mouse. We find Npy expression in NDNF+ cluster in both human and mouse L1.

(E) Ion channels genes. Human clusters show overall low expression of K^+ channels, except for Kcn2, which is highly expressed by NDNF+ cells. Mouse presents higher Kcnip1 and Kcnip4 expressions in all three clusters compared with human. Sodium and chloride channels expression is comparable between the two species.

L1 interneuron subtypes are also differentially regulated by neuromodulation. For example, VIP+ interneurons express 5-hydroxytryptamine receptor 3A (5-HT3AR) and respond to serotonin modulation, whereas NDNF+ interneurons do not.[110] Apart from the direct influence, neuromodulatory inputs may also indirectly affect L1 interneuron activity by adjusting the postsynaptic response of top-down inputs onto L1 interneurons.

In vivo activity of L1 interneurons

L1 interneurons respond to a wide range of sensory and behavioral variables *in vivo*, including locomotion, sensory stimuli, other sensory modalities, and attention. These results are consistent with anatomical studies that show L1 interneurons are primarily innervated by diverse long-range inputs from multiple cortical regions, higher-order thalamus, as well as neuromodulatory centers. By contrast, they receive fewer excitatory inputs from the local cortical region and are weakly driven by bottom-up pathways.[22,43,47,83,111] As a result, when bottom-up and top-down inputs are both present, they are more responsive to active behavior states than sensory information.[111–113]

L1 interneurons, as a whole, respond to a multitude of signals. However, the response of individual L1 interneurons is highly heterogeneous. Each of them not only shows differential selectivity toward the type of inputs but also shows diverse responses to a single stimulus. For instance, a quarter of L1 interneurons in V1 respond to both visual stimuli and locomotion, whereas half of L1 interneurons respond to either visual stimuli or locomotion exclusively.[112] Although the underlying factors have not been thoroughly investigated, the different subtypes of L1 interneurons, the various afferent inputs they receive, and the mutual inhibition among NDNF+ interneurons likely all contribute to the observed heterogeneity of L1 interneuron responses. However, because L1 interneurons produce non-selective inhibition, it remains unclear whether they produce widespread (or broad) inhibition collectively or whether the heterogeneous inhibition is transmitted downstream, increasing the variability of pyramidal neuron responses.

Taken together, despite being a small population, L1 interneurons are transcriptomically diverse and composed of multiple subtypes, each of which possesses unique afferent and efferent connectivities. The most prominent subtype is NDNF+ NGF cells that exert long, strong, and non-selective inhibition, even onto each other. L1 interneurons are most responsive during active behavior states. Individually, each of them shows heterogeneous responses *in vivo*. Overall, being the sole inhibitory inhabitants, L1 interneurons scatter in the dense web of neuronal processes, controlling the flow of information at every crossroad.

CIRCUIT INTEGRATION IN L1

Having thoroughly examined all the key elements within L1, we will now delve into how these components together orchestrate L1 function. In this section, beginning from the perspective of the incoming afferent signals within L1, we plan to review processing following the rough sequence of their recruitment. This is done with the recognition that these signals eventually impact the dendrites of pyramidal neurons and allow the integration of signals received at other compartments of these same pyramidal neurons, resulting in the reconciliation of top-down and bottom-up inputs.

Directing and sorting: Inputs to L1 selectively target pyramidal neurons

L1 is populated by the distal dendritic tufts of pyramidal neurons whose cell bodies are located in other layers, particularly in L2/3 and L5. Inputs to L1 are channeled to converge on the dendritic branches of particular pyramidal neuron tufts. For example, both vM1 and POm synapses are interspersed on dendritic branches in L1 of S1BF in mini-clusters.[114,115] The precise organization of synapses from different inputs on the dendritic tufts is likely meaningful because different parts of the dendritic tufts within L1 may exhibit distinct biophysical properties due to their asymmetrical structure. More distal sites on the branch tend to have smaller diameters and are closer to the extremities, resulting in higher local input resistance.[116] Furthermore, the ion channel composition may also vary along the tuft branches. Therefore, investigating how synapses from individual L1 inputs are distributed across the finer segments of dendritic tufts could provide valuable insights into how pyramidal neurons process each input for subsequent integration.

On the other hand, different inputs to L1 can also be directed onto different subtypes of pyramidal neurons, sorting the incoming information into distinct downstream circuits. One example is shown in RSP, where there are two types of L2/3 pyramidal neurons characterized by their distinct electrophysiological features: regular spiking and low rheobase. Regular-spiking pyramidal neurons have dendritic tufts predominantly located in lower L1, whereas low-rheobase pyramidal neurons have dendritic tufts extending into upper L1. These morphological differences allow for selective recruitment by different inputs to L1. For example, anterior thalamus predominantly targets upper L1 and recruits low-rheobase pyramidal neurons, whereas claustral and ACC inputs primarily target lower L1 and recruit regular-spiking pyramidal neurons.[82,117]

It is important to note that although pyramidal neurons have dendrites in L1 close to various inputs, they do not passively receive all the inputs within their reach. Instead, they show selectivity toward both the type of incoming inputs and the location of the synapses.[118] Supporting this, previous work in RSP has demonstrated that although V1, M2, and LD all project to L1, L5b pyramidal neurons only selectively receive LD input on their dendritic tufts in L1. They receive V1 and M2 inputs on their basal dendrites and proximal trunks near L5, respectively.[118]

Together, these data suggest that L1 does not simply function as a hub where information is broadcast indiscriminately. Instead, it works as a well-ordered network, capable of selectively directing and sorting various information to different downstream targets. These findings illustrate that the precise connections formed between L1 inputs and pyramidal neuron dendrites are selective for the input source, the subtype of pyramidal neurons, the specific dendritic compartment, and even different sites of the dendritic tufts. We believe that future

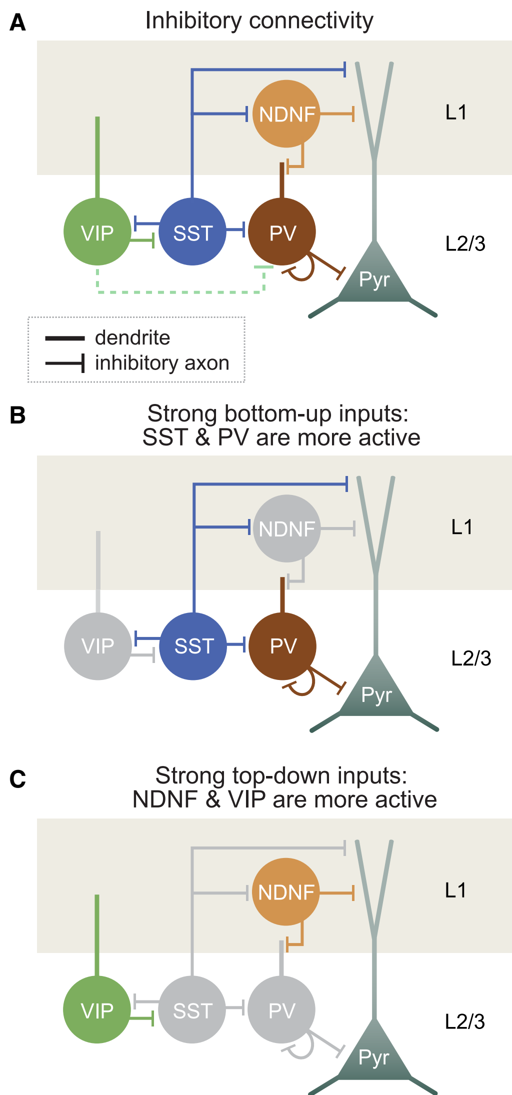

Figure 4. Inhibition in L1

(A) The diagram illustrates the different types of inhibitory neurons and their connectivity within L1 and L2/3. The diagram highlights NDNF+ interneurons in L1, the major type within the L1 interneuron population. The dashed line indicates that the VIP+ to PV+ interneuron connection is different across cortical areas, for example, it does not exist in V1.[124] It remains unclear whether NDNF+ L1 interneurons inhibit VIP+ interneurons.

(B) In the case of a strong sensory stimulus, SST+ interneurons, primarily driven by local excitatory neurons, exhibit increased activity (indicated by blue color). This subsequently inhibits (indicated by gray color) other interneurons, including NDNF+ L1 interneurons.[43] Meanwhile, PV+ interneurons are also activated (indicated by brown color).[125,126]

(C) When the top-down and neuromodulatory inputs are strong, NDNF+ L1 interneurons are activated (indicated by orange color), which subsequently inhibit (indicated by gray color) soma-targeting PV+ interneurons in L2/3.[43] Meanwhile, VIP+ interneurons may also become activated (indicated by green color), which subsequently inhibit (indicated by gray color) dendritic-targeting SST+ interneurons.[43,63,127]

Pyr, pyramidal neurons.

research will unravel the rules that govern these precise connections and the functional implications of such organizations.[119,120]

Gating incoming information: Dendritic inhibition in L1

Inputs to pyramidal neuron tufts in L1 are mainly gated through the recruitment of NDNF+ L1 interneurons and Martinotti-type of SST+ interneurons. These SST+ interneurons have their somata located outside of L1 but possess extensive axonal arborizations in L1.

NDNF+ interneurons produce widespread inhibition onto the distal dendritic tufts of pyramidal neurons. They gate L1 dendritic activity through feedforward inhibition. For instance, VM, which regulates arousal, targets both NDNF+ interneurons and L5 pyramidal-tract (PT) neurons in PFC. NDNF+ interneurons synapse on the apical dendrites of L5 PT neurons and suppress VM-evoked dendritic activity, thereby gating the long-range feedback loops between the thalamus and the neocortex.[121] Moreover, distal dendritic inhibition by NDNF+ interneurons in L1 contributes to the reduction of cross-trial variability and regulates the robustness of sensory-evoked responses in pyramidal neurons *in vivo*.[47]

NDNF+ interneurons also inhibit other inhibitory interneurons, including themselves, as well as L2/3 parvalbumin-expressing (PV+) interneurons, whose dendrites are in L1 (Figure 4A). When NDNF+ L1 interneurons are activated, the perisomatic inhibition of L2/3 pyramidal neurons by PV+ interneurons is lifted, whereas the dendritic tufts in L1 are inhibited by NDNF+ L1 interneurons. This disinhibitory circuit may contribute to fear-conditioning in the neocortex.[45,87,111,122] In addition, the wide presence of presynaptic $GABA_B$ receptors in L1 allows NDNF+ interneurons to modulate their own axon terminals as well as the presynaptic inputs.[107,123]

Unlike most L1 interneurons, the inhibition from Martinotti-type SST+ interneurons is through classical synapses with faster kinetics.[43] Martinotti-type SST+ interneurons primarily receive local excitatory inputs and provide feedback inhibition onto the distal dendrites of pyramidal neurons in L1.[128,129] In contrast to the non-selective inhibition from NDNF+ interneurons, different subtypes of SST+ interneurons form reciprocal connections with selective types of pyramidal neurons and preferentially synapse onto different dendritic compartments of pyramidal dendrites.[130] SST+ interneurons have been implicated in many functions. In L2/3 of V1, they receive horizontal excitatory inputs and play a crucial role in lateral inhibition, by which an activated excitatory neuron suppresses the activity of its neighbors.[125] Furthermore, SST+ interneurons function to maintain dendritic branch-specific activity of pyramidal neurons in the primary motor cortex, after mice learned different motor tasks.[131]

As such, the two sources of inhibitory inputs in L1 each provide unique forms of dendritic inhibition while also influencing each other. Martinotti-type SST+ interneurons can inhibit almost any other interneuron, including L1 NDNF+ interneurons (Figure 4A).[69,124] In the auditory cortex, SST+ interneurons show increasing responses to larger sound amplitudes, whereas NDNF+ interneurons show reduced responses, likely caused by feedforward suppression from activated SST+ interneurons.[43,124] Therefore, in the presence of strong bottom-up inputs, SST+ interneurons are activated by local pyramidal neurons, leading to selective feedback inhibition, specifically at the distal dendrites in L1. Simultaneously, the non-selective

Table 1. Anatomy abbreviations

Acronym	Region
A1	primary auditory cortex
ACC	anterior cingulate cortex
AL	anterolateral area
cV1	contralateral primary visual cortex
dLGN	dorsal lateral geniculate nucleus (of the thalamus)
ECt	entorhinal cortex
HVAs	higher visual areas
LD	lateral dorsal nucleus (of thalamus)
LM	lateromedial area
LP	lateral posterior nucleus (of the thalamus)
M2	premotor cortex
MD	medial dorsal nucleus (of the thalamus)
MGBv	ventral medial geniculate body (of the thalamus)
ORB	orbital cortex
PFC	prefrontal cortex
PM	posteromedial area
POm	posterior medial nucleus (of the thalamus)
PRh	perirhinal cortex
RL	rostrolateral area
RSP	retrosplenial cortex
S1	primary somatosensory cortex
S1BF	primary somatosensory cortex barrel field
S2	secondary somatosensory cortex
TEa	temporal association areas
V1	primary visual cortex
VPM	ventral posteromedial nucleus (of the thalamus)
VM	ventromedial nucleus (of the thalamus)
vM1	vibrissal primary motor cortex

dendritic inhibition in L1 mediated by NDNF+ interneurons is reduced (Figure 4B). In contrast, during active behavior states, when top-down inputs are predominant, NDNF+ interneurons are activated, tipping the scale toward inhibition driven by L1 interneurons (Figure 4C). This dynamic recruitment of different forms of inhibition under distinct conditions demonstrates the crucial role L1 plays in the precise regulation of dendritic activity during different behavior contexts.

The subtlety of these mechanisms is also regulated by differences in where on the dendritic tufts NDNF+ or SST+ interneurons target in L1. SST+ interneurons have been found to be biased toward synapsing onto dendritic spines, whereas indirect evidence suggests that NDNF+ interneurons may preferentially target dendritic shafts.[132,133] This may further contribute to the synergetic impact of dendritic inhibition on regulating dendritic excitability. Future investigation examining the organization principles of both excitatory and inhibitory inputs on the fine dendritic tufts could allow us to better appreciate the level of precision in the structural and functional organization of L1.

Together, the two forms of inhibition in L1 allow for dynamic control of information flow at various temporal and spatial scales (Table 2). This ranges from broad and slow inhibitory control by NDNF+ interneurons to selective gating of dendritic branches by Martinotti-type of SST+ interneurons.

Modulating tuft excitability with neuromodulation

Neuromodulation can modulate tuft excitability by directly acting on pyramidal neurons. In addition to changes in dendritic excitability mediated through ionotropic channels located along the dendrites, neuromodulatory inputs can indirectly regulate dendritic excitability by recruiting dendritic inhibition.[134–136] For instance, cholinergic inputs can drive both VIP+ interneurons and L1 interneurons according to the behavioral state of the animal. During active whisking or locomotion, ACh activates VIP+ interneurons and alleviates inhibition from SST+ interneurons, leading to disinhibition and increased excitability at the dendritic tufts of pyramidal neurons. Aversive stimuli such as foot shock and airpuff can recruit a large number of L1 interneurons, providing strong inhibition onto the dendritic tufts of pyramidal neurons (Figure 4C).[45,87,93,137–139] Moreover, neuromodulation can act through metabotropic receptors and adjust excitability bidirectionally depending on the activity of the neuron. One study using slice physiology found that cholinergic signals, mediated by Gq-coupled muscarinic ACh receptor M1, effectively suppress the activity of L1 NGF cells when they are actively firing action potentials, indicating that its effect on L1 interneurons may be activity dependent.[140] Together, neuromodulation provides further regulation of the dendritic excitability, fine-tuning the impact of L1 inputs.

Integration and plasticity: Dendritic spikes on the tufts

Synaptic inputs conveyed to the dendrites can be processed nonlinearly and transformed into dendritic spikes, including sodium-, NMDA-, and calcium receptor-mediated spikes.[141,142] These dendritic spikes are typically confined to the specific dendritic compartment where they originate, resulting in distinct postsynaptic effects in response to inputs received elsewhere. This is due to ion channels being selectively distributed within different dendritic compartments. Dendritic spikes are distinct in terms of their amplitude, duration, and propagating distance, making each uniquely suited for modulating input currents received at different dendritic compartments of pyramidal neurons. Although inputs onto the thin dendrites, including tuft, oblique, and basal dendrites, generate NMDA spikes, inputs closer to the trunks induce calcium spikes.[143–148] In contrast to calcium spikes, which propagate several hundred micrometers, NMDA spikes are much more local and spatially restricted.[148]

NMDA spikes, in particular, contribute to synaptic integration and synaptic plasticity, which are important for various aspects of cortical function, including sensory-motor integration and learning.[12,25,43,61,123,136,148,149] In L1, NMDA spikes, also known as NMDA plateau potential, can be generated at the dendritic tufts, resulting in prolonged depolarization at the generation sites.[144] This sustained depolarization in the distal dendrites enhances synaptic integration and allows the pyramidal neurons to summate inputs from different sites over an extended period. In sensory cortices, when the top-down inputs at tufts in L1 and the

Table 2. Different types of inhibition in L1

Characteristic	Inhibition from NDNF+ L1 interneurons	SST+ Martinotti interneurons
Type of inhibition	feedforward inhibition	feedback inhibition
Signal transmission	unclear *NGF: non-specific form ("volume transmission")*	classical synaptic
Response kinetics	slow	fast
Inhibitory scope	broad	specific
Mutual inhibition	yes	no
Primary activation source	top-down and neuromodulatory inputs	local excitatory neuron
Preferred target site on tufts	unknown	dendritic spines

bottom-up inputs at the basal dendrites and somatic areas are received coincidently or within a short temporal window, their signals can be integrated in a nonlinear manner.[150] In L2/3 pyramidal neurons, this process is associated with enhanced L1 influence on the action potentials generated at the soma, resulting in increases in the neural output of pyramidal neurons.[151,152] Together, the presence of these dendritic spikes may enable the integration and interaction of top-down information within L1 with incoming information onto other compartments of pyramidal neurons. This integration could occur in both spatial and temporal dimensions, facilitating the coordination of different streams of information. In addition, NMDA spikes driven by L1 inputs can mediate synaptic plasticity, such as synaptic long-term potentiation, even in the absence of somatic spikes.[143,153,154] For example, rhythmic whisker stimulation induces long-term potentiation in L2/3 pyramidal neurons, and POm-mediated NMDA spikes in L1 are required for this phenomenon.[153] These NMDA spikes are also associated with experience-dependent reorganization of cortical sensory maps.[142,155] It would be interesting to further investigate the role of NMDA spikes on the distal dendritic tufts in L1.

In summary, we have discussed several aspects of L1 function and showcased how different elements collaborate to accomplish each task. When incoming information enters L1, it is directed, sorted, gated, modulated, and finally integrated in a pyramidal neuron. All these steps are achieved through coordinated actions from the conglomerate of different components that meet in L1. Overall, the interplay between excitation, inhibition, and neuromodulation enables the precise and dynamic processing of incoming signals in L1 that is crucial for cognitive functions throughout the neocortex, such as behavioral state- or context-dependent sensory perception, sensory-motor integration, synaptic plasticity, and learning.[156–158]

L1 MAY BE IMPACTED IN THE DISEASE STATES

Changes in L1 have often been overlooked when investigating the pathology of various diseases that impact the nervous system. We believe that understanding the role of L1 is crucial for advancing our understanding and treatment of brain diseases.

The impact on neuronal structure and function is the primary cause of brain disease. We suspect L1's proximity to the pia makes it particularly vulnerable to defects in non-neuronal processes, such as neuroinflammation and major blood circulation (Figure 5). The meninges, which consist of the dura mater, arachnoid mater, and pia mater, form protective layers around the brain parenchyma. They contain a diverse population of immune cells, which are increasingly acknowledged for their crucial roles in the central nervous system.[159] Some of these immune cells can penetrate the brain parenchyma, likely interacting with the L1 environment. Interestingly, T-cell-derived interferon-γ (IFN-γ) can activate L1 interneurons, and this process is important for normal social behaviors, indicating the link between meningeal immunity and neuronal activity via L1 circuit.[160] Additionally, the blood-brain barrier, in conjunction with the impermeable properties of the arachnoid mater and pia mater, helps safeguard the brain parenchyma in healthy conditions.[161] However, these protective properties may be compromised in the presence of inflammation or physical challenges, potentially affecting the L1 environment. Conditions such as stroke, infection, and neurodegenerative diseases could impact the integrity of L1 due to its proximity to the affected areas.[162] The dysfunction of the meninges may result in the potential increase in meninge resident cells present in L1, leading to a dysregulated immune environment in the brain. Furthermore, the permeable barriers would fail to protect L1 from the invasion of threats such as virus and bacteria, and other non-local immune cells from the circulation, making it particularly susceptible. Such changes in the L1 environment will likely activate its resident glial cells, such as astrocytes and microglia, eventually affecting the neuronal structure and activities in the brain.

Late maturation of L1 circuits and its impact on brain function

In multiple sensory modalities, prenatal and postnatal spontaneous activity shapes the initial connections, whereas sensory-evoked activity is essential for the refinement and maturation of these inputs.[163] Soon after sensory exposure, sensory feature detection is strengthened and finely tuned following the maturation of inhibition. However, circuits involved in conscious perception of sensory inputs, attentional control, and cognitive processing require top-down feedback modulation of sensory inputs. Studies have suggested that early in development, the brain is primarily driven by bottom-up inputs, and the formation of feedback connectivity gradually develops during later stages.[164,165] L1 ultimately serves as a vital center for receiving

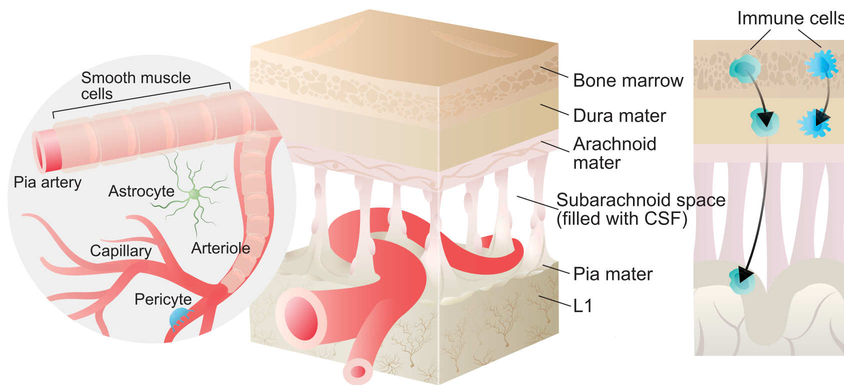

Figure 5. The proximity of L1 to pia structures
The illustration highlights the proximity of L1 to pia structures. (Middle) Above L1 resides the pia mater, arachnoid mater, dura mater, and the bone marrow. The subarachnoid space between the pia mater and arachnoid mater consists of the arachnoid trabeculae and the major blood vessels, with cerebrospinal fluid (CSF) filling the space. (Right) L1 is in close proximity to structures outside of the brain parenchyma, such as bone marrow and dura mater, which are enriched with immune cells (green and blue). Some of these immune cells (green on the left) can enter the brain parenchyma. (Left, circle panel) Magnified view of the vessels above and in L1, including the pial artery and descending arterioles that are surrounded by smooth muscle cells, and the capillaries that are surrounded by pericytes. Astrocytes have their endfeet on all the vessels. The figure is adapted from Kaplan et al.[161] and Castellani et al.[159]

top-down inputs, but some of these connections mature late in development and are likely dependent upon sensory experience. For example, M2/ACC input to NGF cells in mice V1 only matures after eye opening and is dependent upon ascending visual inputs.[22] Moreover, the distal dendrites of pyramidal neurons also mature gradually, facilitating proper dendritic integration of long-range excitatory and local inhibitory connectivity in L1.[166–168] In the auditory cortex of gerbils, it has been demonstrated that short phases of sensory deprivation can influence perceptual learning. This, too, suggests that sensory experiences play a pivotal role in the maturation of top-down inputs, which modulate sensory circuits and are crucial for learning and decision making.[169] Importantly, this modulation was found to be highly dependent on inhibition in the auditory cortex. Similarly, based on invasive electrophysiological recordings in cochlear-implanted deaf cats, it was hypothesized that delayed sensory experience following birth particularly affects the elaboration of top-down processing.[170,171] Taken together, these studies suggest that sensory experiences not only fine-tune our perceptions but also play an indispensable role in the maturation of top-down circuits. Although it remains unknown whether L1 interneuron-mediated inhibition is involved, impairments in L1 synaptic function and connectivity, such as improper sensory stimulus during adolescence seem likely candidates to contribute to the development of neuropsychiatric and neurodevelopmental disorders.[141,172] In addition, L1 interneurons express numerous genes that are associated with neuropsychiatric diseases and neurodevelopmental disorders (Figure 3).[173] Many risk genes implicated in neurodevelopmental disorders such as autism spectrum disorder (ASD) function within dendrites of pyramidal neurons and are important for synaptic function in L1.[174]

Together, this evidence highlights the importance of future research in examining the development of L1 circuits and the contribution of L1 in nervous system disorders.

CONCLUSIONS AND FUTURE OUTLOOK

By rethinking the traditional feedforward ordering of information flow in the neocortex, the significance of L1 organization has become evident. Rather than a feedforward network by which information travels unidirectionally to higher-order cortical regions, the complex flow of top-down and neuromodulatory inputs demonstrates a series of nested loops that ultimately converge back to L1. Adding complexity to the modes by which information travels, different outcomes are likely dependent on both the timing and the precise synaptic position of inputs to their target cells. Nowhere is this truer than in L1, where various inputs seemingly lead to contrasting outcomes. For example, the SST+ interneuron axons in L1 can both simultaneously restrict the targeted pyramidal cell inputs through inhibition while increasing excitation through disinhibiting NDNF+ interneurons. Furthermore, the outcome of these interactions is dependent not only on the timing of excitation or inhibition but also on the state-dependent recruitment of neuromodulatory inputs that can be affected by attention, locomotion, or arousal. Moreover, L1 signaling can also change as a function of the network activation that precedes it. For instance, previous events can drastically alter activity because of dendritic plateau potential, shifts in the activation of NMDA signaling that is enriched in upper L1, or activation of proximally located calcium channels. Hence, the net result of L1 activation depends upon the specific inputs activated, their timing of recruitment, and the set state of L1 based on previous activities. Together, this allows L1 to function dynamically to reconcile the outcome based upon both past and present circumstances.

Although we have here discussed in considerable detail how this impacts sensory regions, particularly V1, L1 functions across motor, premotor, and higher association cortical areas. Given the enormous flexibility in excitatory, neuromodulatory, and inhibitory signals, it is not surprising how this diversity could, with differences in inputs, connectivity, timing, neuromodulation, or intrinsic excitability, be drastically adjusted to function differentially across the breadth of distinct cortical regions. This flexibility no doubt explains how the gross homogeneity of the neocortex can be specialized to mediate the wide array of cognitive functions within the cerebrum. Hence, it becomes less surprising to consider how the properties of L1 could also subserve additional modalities as disparate as motor control and higher cognition function. Furthermore, given the complexity of the connectivity within L1, the question of how it is assembled during development across the neocortex will pay considerable dividends in helping us understand mature function. Hence, by

following the dual path of examining how L1 acts in different cortical regions and how development shapes them, there lies a path for understanding how L1 allows for the alignment of the external world with our internal perceptions. Additional areas worth considering are how the collateral projections to L1 and infragranular layers function together, how evolutionary changes could adjust brain function, and how L1 dysfunction could contribute to neurological and neuropsychiatric disorders. Understanding both the variation and nuance of how L1 function acts within rodents versus primates will provide both insight and surprises into how L1 has adapted across species and how this has allowed for higher cognitive abilities in humans.

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AUTHOR CONTRIBUTIONS

S.H., L.A.I., and G.F. wrote the original draft, and S.H. created visualizations. G.S. and L.A.I. created gene expression plots in Figure 3. S.J.W., L.A.I., and G.F. provided critical review and editing of the manuscript. All authors contributed to the conceptualization and final editing.

DECLARATION OF INTERESTS

G.F. is a founder of Regel Therapeutics, which has no competing interests with the present manuscript, and an advisor for the McKnight Foundation, *Neuron*, and *Annual Review of Neuroscience*.

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