

Multisensory Integration in Pyramidal Cell Dendrites in Parietal Cortex of Awake Behaving Mice

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Abbreviation List:

2AFC: two-alternative forced-choice task

2P: 2Photon Calcium imaging

A.U. : Arbitrary Unit

AP: Antero-Posterior

Area AL: anterolateral visual area

bAPs: Backpropagating action potentials

Ca²⁺: Calcium

Camk2 α : calcium-calmodulin-dependent kinase II

Cpd: Cycles Per Degree

DAPI: 4',6-Diamidino-2-Phenylindole

DV: Dorsoventral

fMRI: functional Magnetic Resonance Imaging

GECIs: Genetically Encoded Calcium Indicators

GEVIs: Genetically Encoded Voltage Indicators

GRASP: GFP Reconstitution Across Synaptic Partners

IPS: Intraparietal Sulcus

ITI: Inter-Trial Interval

L: Lateral

L1: Layer 1

L2/3: Layer 2/3

L5: Layer 5

LGN: Lateral Geniculate Nucleus

LP: Lateral Posterior nucleus

MEAT: Multisensory Evidence Accumulation Task

MS: Multisensory

MSI: Multisensory Integration

Na⁺: Sodium channels

NMDA: N-methyl-D-aspartate

PBS: Phosphate-Buffered Saline

PFA: Paraformaldehyde

RL: Rostrolateral visual area

ROI: Regions of Interest

S1: Primary Somatosensory cortex

SC: Superior Solliculus

SEM: Standard Error of the Mean

STDP: Spike-Timing-Dependent Plasticity

STSp: Posterior Superior temporal Sulcus

tetO: tetracycline-responsive regulatory element

Th: Thalamus

tTA: tetracycline-controlled transactivator protein

V1: Primary Visual cortex

VPL: Ventral Posterolateral nucleus

VPM: Ventral Posteromedial nucleus

Summary:

The brain's remarkable ability to integrate information from multiple sensory modalities is known as multisensory integration (MSI). MSI is fundamental for creating a coherent perception of the world and guiding adaptive behavior. While the behavioral advantages of MSI, such as enhanced detection and discrimination, are well established, the underlying neural computations, particularly at the cellular and subcellular levels within the neocortex, remain largely unresolved. This thesis investigated the anatomical substrates and functional mechanisms of visuotactile integration in the mouse brain, focusing on the rostrolateral visual area (RL), a region implicated as a parietal association hub. We specifically examined the role of Layer 2/3 (L2/3) pyramidal neurons and their dendrites, hypothesizing that dendritic computations are critical for implementing MSI and are subject to modification through learning.

Functionally, we investigated neural correlates of MSI using behavioral paradigms combined with chronic widefield calcium imaging. Mice trained on a visuotactile evidence accumulation task exhibited significant behavioral benefits during multisensory trials, demonstrating enhanced perceptual sensitivity compared to unisensory conditions. Initial population-level imaging using widefield calcium recordings confirmed RL as a key integration hub, displaying enhanced, often supra-linear, population activity during multisensory trials that correlated with improved behavioral performance.

To delineate the circuitry supporting visuotactile integration in RL, we employed viral tracing techniques. Retrograde tracing confirmed that RL receives convergent input from primary visual (V1) and somatosensory (S1) cortices, as well as thalamic nuclei. Quantitative analysis revealed the highest density of projecting somata originated from V1, followed by S1, with significantly lower densities from thalamic sources, highlighting the importance of direct cortico-cortical pathways. Dual anterograde tracing provided crucial insights into the laminar organization of these inputs within RL. We discovered a striking segregation: S1 projections predominantly targeted superficial layers (L1/L2/3), the principal receiving zone for apical dendrites of L2/3 neurons, while V1 projections primarily innervated mid-to-deep layers. This anatomical arrangement provides a compelling structural basis for modality-specific

inputs interacting with distinct dendritic compartments, potentially enabling non-linear integration.

Subsequent 2-Photon calcium imaging at single-cell resolution during a similar discrimination task further substantiated RL's role, revealing distinct populations of neurons responsive to visual, tactile, or multisensory stimuli. However, simple averaging of somatic responses from functionally classified cells did not always reveal clear supra-linear summation, highlighting the need for subcellular investigation and analysis of learning-induced changes.

A central aim was to probe MSI at the dendritic level. We successfully established methodologies combining sparse neuronal labeling with chronic, simultaneous 2-Photon calcium imaging of somatic and apical dendritic activity in L2/3 pyramidal neurons of V1 and RL in awake, behaving mice.

Using this longitudinal imaging approach, we compared neuronal responses during passive sensory exposure before (naive) and after (trained) associative learning in a detection task. This revealed profound learning-induced plasticity, particularly within RL. Both dendritic and somatic responses to tactile and visuotactile stimuli were dramatically amplified in RL following training, while responses in V1 and responses to visual stimuli showed less pronounced changes.

These functional plasticity findings, particularly the strong enhancement of tactile responses in RL, align remarkably well with our anatomical data showing dense S1 input specifically targeting the superficial layers of RL where the apical dendrites of L2/3 neurons reside. This convergence strongly suggests that associative learning potentiates synaptic inputs onto these dendritic compartments. While the complete analysis of dendritic dynamics during active task performance is ongoing, the current data provide substantial evidence for experience-dependent modulation of sensory processing at both somatic and dendritic levels.

In conclusion, this thesis provides a multiscale investigation into the anatomical basis and functional implementation of visuotactile integration and learning in the mouse parietal association cortex. We demonstrated the convergence of visual and somatosensory pathways onto RL with distinct laminar targeting, providing a structural

substrate for integration. We confirmed RL's role as an integration hub where population activity correlates with better performance in MSI-dependent behavioral tasks. Most critically, we revealed profound learning-induced plasticity within RL, characterized by a dramatic amplification of tactile and multisensory responses in L2/3 neurons, observable at both the somatic and dendritic levels. By establishing methods for subcellular imaging in behaving animals and linking anatomical connectivity with functional plasticity, this work strongly supports the hypothesis that experience-dependent modulation of dendritic integration within associative cortical areas like RL is a key mechanism underlying the brain's ability to effectively combine information across senses to guide perception and behavior. Future research completing the analysis of active dendritic processing and employing targeted manipulations will further elucidate the precise computational roles of dendrites in multisensory perception and learning.

Zusammenfassung

Die bemerkenswerte Fähigkeit des Gehirns, Informationen aus unterschiedlichen Sinnesmodalitäten zu integrieren, wird als multisensorische Integration (MSI) bezeichnet. MSI ist fundamental wichtig für die Erzeugung einer kohärenten Wahrnehmung der Welt und die Steuerung adaptiven Verhaltens. Obwohl die Verhaltensvorteile von MSI, wie verbesserte Detektion und Diskrimination, gut etabliert sind, bleiben die zugrunde liegenden neuronalen Berechnungen, insbesondere auf zellulärer und subzellulärer Ebene im Neokortex, weitgehend ungeklärt. Diese Doktorarbeit untersuchte die anatomischen Substrate und funktionellen Mechanismen der visuotaktilen Integration im Mausgehirn, wobei der Fokus auf dem rostrolateralen visuellen Areal (RL) lag, einer Region, die als parietaler Assoziationsknotenpunkt gilt. Wir untersuchten spezifisch die Rolle von Pyramidenneuronen der Schicht 2/3 (L2/3) und deren Dendriten, mit der Hypothese, dass dendritische Berechnungen entscheidend für die Implementierung von MSI sind und durch Lernen modifiziert werden können.

Um die Verschaltung zu charakterisieren, die die visuotaktile Integration in RL unterstützt, setzten wir virale Tracing-Techniken ein. Retrogrades Tracing bestätigte, dass RL konvergente Eingänge vom primären visuellen (V1) und somatosensorischen (S1) Kortex sowie von Thalamuskernen erhält. Quantitative Analysen zeigten, dass die höchste Dichte projizierender Somata aus V1 stammte, gefolgt von S1, mit signifikant geringeren Projektionen aus thalamischen Quellen, was die Bedeutung direkter kortiko-kortikaler Bahnen hervorhebt. Duales anterogrades Tracing lieferte wichtige Einblicke in die laminare Organisation dieser Eingänge innerhalb von RL. Wir entdeckten eine auffällige Segregation: S1-Neurone projizierten überwiegend auf oberflächliche Schichten (L1/L2/3), die Hauptempfangszone für apikale Dendriten von L2/3-Neuronen, während V1-Projektionen primär mittlere bis tiefe Schichten innervierten. Diese anatomische Anordnung bietet eine strukturelle Grundlage für die Interaktion modalitätsspezifischer Eingänge mit unterschiedlichen dendritischen Kompartimenten, was potenziell nicht-lineare Integration ermöglicht.

Funktionell untersuchten wir neuronale Korrelate von MSI mithilfe von Verhaltensparadigmen in Kombination mit chronischem Calcium-Imaging. Mäuse, die auf eine visuotaktile Evidenzakkumulationsaufgabe trainiert wurden, zeigten

signifikante Verhaltensvorteile während multisensorischer Durchgänge, was eine verbesserte perzeptuelle Sensitivität im Vergleich zu unisensorischen Bedingungen demonstrierte. Initiale Bildgebung auf Populationsebene mittels widefield-calcium-imaging bestätigte RL als zentralen Integrationsknotenpunkt, der während multisensorischer Durchgänge eine verstärkte, oft supra-lineare Populationsaktivität zeigte, die mit verbesserter Verhaltensleistung korrelierte. Nachfolgendes Zwei-Photonen-Calcium-Imaging mit Einzelzellauflösung während einer ähnlichen Diskriminationsaufgabe untermauerte die Rolle von RL weiter und offenbarte klar unterscheidbare Populationen von Neuronen, die auf visuelle, taktile oder multisensorische Stimuli ansprachen. Jedoch zeigte die einfache Mittelung somatischer Antworten von funktionell klassifizierten Zellen nicht immer eine klare supra-lineare Summation, was die Notwendigkeit für subzelluläre Untersuchungen und die Analyse lerninduzierter Veränderungen unterstrich.

Ein zentrales Ziel war es, MSI auf dendritischer Ebene zu untersuchen. Wir kombinierten erfolgreich eine Sparse-Labeling-Methode mit chronischem, simultanem Zwei-Photonen-Calcium-Imaging somatischer und apikaler dendritischer Aktivität in L2/3-Pyramidenneuronen von V1 und RL in wachen, sich verhaltenden Mäusen.

Mithilfe dieses longitudinalen Imaging-Ansatzes verglichen wir neuronale Antworten während passiver sensorischer Exposition vor (naiv) und nach (trainiert) assoziativem Lernen in einer Detektionsaufgabe. Dies offenbarte stark ausgeprägte lerninduzierte neuronale Plastizität, insbesondere innerhalb von RL. Sowohl dendritische als auch somatische Antworten auf taktile und visuotaktile Stimuli wurden nach dem Training in RL dramatisch verstärkt, während Antworten in V1 und Antworten auf visuelle Stimuli weniger ausgeprägte Veränderungen zeigten.

Diese Ergebnisse zur funktionellen Plastizität, insbesondere die massive Verstärkung taktiler Antworten in RL, stimmen sehr gut mit unseren anatomischen Daten überein, die dichten S1-Input zeigen, der spezifisch auf die oberflächlichen Schichten von RL projiziert, wo sich die apikalen Dendriten von L2/3-Neuronen befinden. Diese Konvergenz legt nahe, dass assoziatives Lernen synaptische Eingänge auf diese dendritischen Kompartimente potenziert. Obwohl die vollständige Analyse der dendritischen Dynamik während aktiver Aufgabenausführung noch andauert, liefern

die aktuellen Daten substantielle Belege für eine erfahrungsabhängige Modulation der sensorischen Verarbeitung auf sowohl somatischer als auch dendritischer Ebene.

Zusammenfassend bietet diese Doktorarbeit eine vielschichtige Untersuchung, der anatomischen Grundlagen und funktionellen Implementierung von visuotaktile Integration und der zugehörigen Lernprozesse im parietalen Assoziationskortex der Maus. Wir demonstrierten die Konvergenz visueller und somatosensorischer Signalwege auf RL, speziell solcher, die im laminaren Bereich ankommen und durch die RL ein strukturelles Substrat für die Integration dieser Signale bildet. Wir haben die Rolle von RL als Integrationszentrum nachgewiesen, in dem Populationsaktivität mit einer Leistungssteigerung in MSI-abhängigen Verhaltensversuchen korreliert. Vorallem konnten wir eine lerninduzierte Plastizität innerhalb von RL zeigen, charakterisiert durch eine Verstärkung taktiler und multisensorischer Antworten in L2/3-Neuronen, die wir sowohl auf somatischer als auch auf dendritischer Ebene beobachten konnten. Durch die Etablierung von Methoden für subzelluläres Imaging in wachen Tieren, welche Verhaltenstest durchlaufen, sowie die Verknüpfung anatomischer Konnektivität mit funktioneller Plastizität unterstützt diese Arbeit nachdrücklich die Hypothese, dass die erfahrungsabhängige Modulation dendritischer Integration innerhalb assoziativer kortikaler Areale wie RL ein Schlüsselmechanismus ist, der der Fähigkeit des Gehirns zugrunde liegt, Informationen über Sinne hinweg effektiv zu kombinieren, um Wahrnehmung und Verhalten zu steuern. Zukünftige Forschung, welche die Analyse aktiver dendritischer Verarbeitung vervollständigt und gezielte Manipulationen einsetzt, wird die Rolle von Dendriten in der Verarbeitung multisensorischer Wahrnehmung und der zugehörigen Lernprozesse weiter aufklären.

1. Introduction

1.1. Sensory Processing in the Neocortex

Daily existence confronts us with a dynamic stream of information originating from diverse and complex environments. Many sensory organs extract individual sensory modalities to make sense of and interact with these environments. Understanding how the brain processes sensory information and drives behavior is currently one of the most important questions in neuroscience. Over the last decades, vast efforts have been devoted to studying how our sensory systems transduce signals and extract useful features about our environment (Hudspeth & Logothetis, 2000). The ability to integrate and process sensory signals into information and actions is fundamental to survival and is a driving force for evolution. However, our perception of the world is rarely unisensory; instead, it arises from the seamless integration of inputs arriving concurrently through multiple sensory channels – vision, hearing, touch, smell, and taste. Nevertheless, the exact mechanism of how those sensory streams converge and where in the brain this takes place to produce these multisensory percepts is still unresolved (Stein & Stanford, 2008).

Determining how sensory perception arises in the brain is not easy. Although, considering that neurons are the brain's functional unit, it might lead us to the key principle of the brain's coordination of perceiving sensory signals and interacting with the world.

At the first level, sensory signals are received by the peripheral nervous system using our senses. These sensory inputs are conveyed to the brainstem, which sends the information to the thalamus and other subcortical areas to reach the neocortex finally (Cappe & Barone, 2005). It is believed that in the neocortex, sensory processing follows a simplistic pathway where information is processed to build complex features of sensory perception. This information is then used to guide decisions and actions. Primary sensory areas represent the initial stage of neocortical processing, serving as

the main recipients of sensory information from the primary thalamic nuclei. These thalamic projections deliver information about the external sensory environment to the primary sensory areas and are categorized as feedforward inputs. As a result, primary sensory areas are highly organized based on specific sensory modalities and specialize in processing sensory signals' physical characteristics. As information progresses through the processing stream to secondary and association areas, a unified percept is formed, leading to sensory perception. However, recent neuroscience advancements have revealed that this view is overly simplistic (Bieler et al., 2017; Ibrahim et al., 2016). With the advent of techniques (imaging, *in vivo* studies, electrophysiology...) it has become evident that primary sensory areas are involved in more complex processes than previously thought (Pasternak & Greenlee, 2005). Recent evidence shows that these primary sensory cortices also engage in higher-order integration and modulation. This process, known as Multisensory Integration (MSI), is not merely the summation of individual sensory streams but rather a sophisticated computation that synthesizes information to create a unified, coherent perceptual experience (Postmes et al., 2014; Stein & Stanford, 2008).

1.2. Multisensory Integration

Multisensory integration is the process by which the brain combines and synthesizes information from multiple sensory modalities (such as vision, sound, touch, taste, and smell) to create a unified and coherent perception of the environment (Postmes et al., 2014). This perception occurs through various specialized sensory organs, each optimized to convert signals from a specific sensory modality. This integration enables us to make sense of complex stimuli and facilitates more accurate and adaptive responses to our surroundings. Integrating information across multiple sensory modalities, enhances our ability to detect and respond to events (Körding et al., 2007). For instance, multisensory integration allows us to perceive a sound as coming from a particular direction based on visual cues or to experience a flavor through the combined input of taste and smell (Stein & Meredith, 1993; Wozny et al., 2010). Consequently,

integrating sensory information into a unified perception of our environment is a fundamental function of the nervous system and underpins our conscious experience. Hence, disruptions in this process are linked to various neurological disorders. For instance, individuals on the autism spectrum often exhibit impairments in multisensory integration, which hampers their ability to combine even basic non-social sensory inputs (Ostrolenk et al., 2019). Conversely, patients with schizophrenia tend to show heightened multisensory integration compared to healthy individuals, which may result in the erroneous integration of unrelated sensory information (Stone et al., 2011). This underscores that multisensory integration is a delicate balancing act, with significant consequences when disrupted. Although the processing of unisensory information in the brain has been a primary focus of neuroscience since its inception, the mechanisms by which this information is integrated across different sensory modalities remain poorly understood. To uncover the causes and mechanisms of neurological disorders and develop effective treatments targeting either symptoms or underlying causes, we must deepen our understanding of how the brain integrates multisensory information in healthy/neurotypical individuals.

1.3. Multisensory enhancement

Multisensory enhancement refers to the simultaneous presentation of stimuli from two or more sensory modalities (e.g., visual and auditory). This leads to a stronger or more efficient response than when the stimuli are presented individually. This enhancement occurs when multisensory integration improves perception, leading to faster, more accurate, or more robust detection of events in the environment (Rowland, 2008).

A classic example of multisensory enhancement is the "McGurk effect", where a mismatch between auditory and visual stimuli creates an altered perception. For instance, when individuals hear the syllable "ba" but see the speaker's lips mouthing "ga," they often perceive the syllable "da," a fusion of the two sensory inputs (McGurk &

Macdonald, 1976). This illustrates how visual and auditory information can interact to produce a perceptual outcome neither modality could generate independently.

In another study, Stein and Meredith demonstrated that multisensory enhancement could improve response times (Stein & Meredith, 1993). Their experiments showed that when both visual and auditory stimuli were presented together, subjects responded faster than when either stimulus was presented alone. This synergistic effect emphasizes the role of multisensory integration in enhancing perceptual processing.

Moreover, multisensory enhancement can take different forms depending on how the brain combines information from multiple senses. The three main types of multisensory integration are superadditive, additive, and subadditive. These types refer to how sensory inputs from various modalities (like vision, hearing, or touch) are processed and combined to enhance perception (Choi et al., 2018; Stein & Stanford, 2008).

a) Superadditive Multisensory Integration:

This occurs when the combined response from two or more senses is greater than the linear sum of the individual responses from each sense alone. Essentially, the brain generates a much stronger perceptual response than would be expected by simply adding up the separate inputs.

If a faint sound and a dim light are presented together, and the combined response (e.g., a faster reaction time or stronger perception) is much greater than the sum of each stimulus alone, this is superadditive enhancement. Superadditive responses typically occur when the individual stimuli are weak or ambiguous. The brain benefits most from combining the sensory inputs to create a clearer, stronger perception (Stevenson & James, 2009).

b) Additive Multisensory Integration:

In additive integration, the combined response equals the sum of the responses to each sense. This means the brain combines the inputs from different senses but doesn't

amplify the result beyond what each sense individually contributes. This means that the response of individual neurons, as measured by their evoked firing rates, is equal to the sum of the responses elicited by each unisensory stimulus component.

If you see a moderate flash of light and hear a moderate sound, the brain's response might equal the sum of both stimuli, with no extra enhancement beyond their combined impact. Additive integration is common when the stimuli are more clear or strong individually. Combining them benefits the brain but doesn't need to over-enhance the perception (Stein & Stanford, 2008).

c) Subadditive Multisensory Integration:

This happens when the combined response is less than the sum of the individual responses, but some integration is still happening. The brain processes the inputs together but may down-regulate the response, especially if the stimuli are already strong and clear. In subadditive integration, the responses are lower than expected from the linear sum of the unisensory responses (Stein & Meredith, 1993).

If you see a bright flash and hear a loud sound simultaneously, your brain might combine the signals, but the overall response could be smaller than if you just added up the reactions to each stimulus. You still benefit from combining the signals, but the brain doesn't need to amplify the response as much because the inputs are already strong. The senses already provide enough clarity, so the combination produces a balanced, but not exaggerated, perception.

The superadditive and the subadditive responses are forms of non-linear integration, while additive responses represent a linear integration.

These forms of multisensory enhancement illustrate how the brain flexibly combines sensory inputs, tailoring the level of integration based on the clarity and strength of the stimuli, allowing us to perceive the world more efficiently and adaptively. Investigating MSI under varying conditions of sensory evidence and ambiguity is therefore crucial for understanding its adaptive function. (**Figure 1**, (Stein & Stanford, 2008))

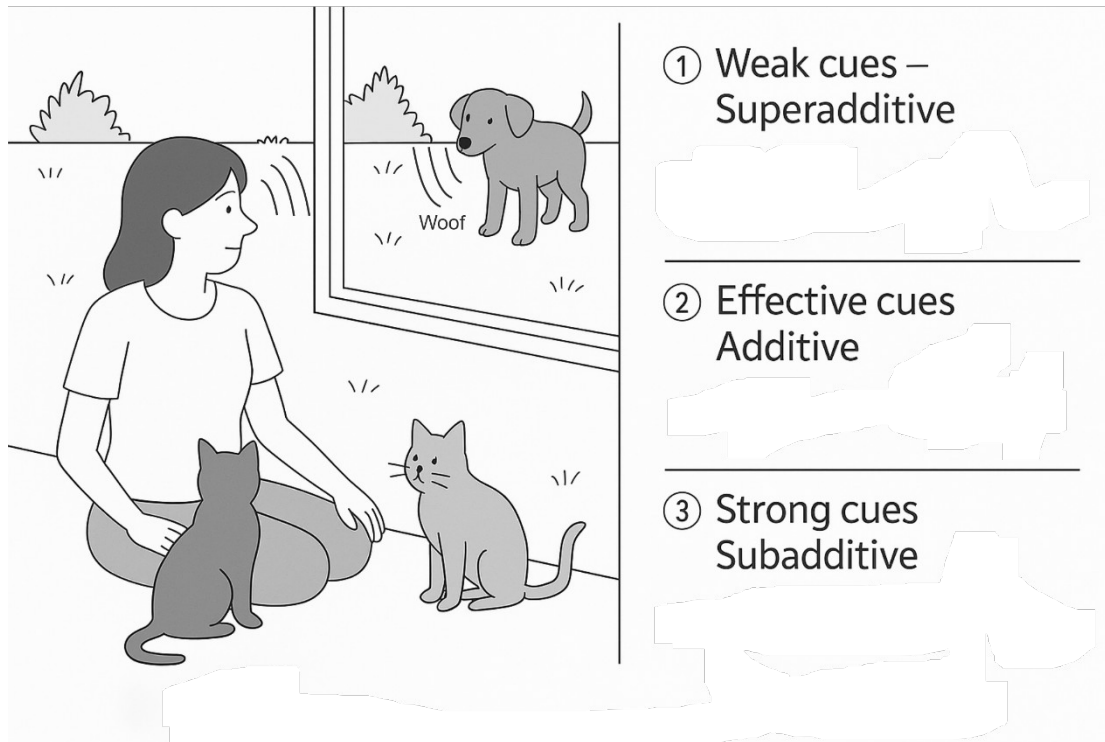


Figure 1: Form of Multisensory enhancement.

This figure illustrates the principle of inverse effectiveness in multisensory integration using the scenario of detecting an approaching dog via sight and sound. When sensory inputs are weak (e.g., dog far away, top panel), combining them yields a superadditive neural response—one that is greater than the sum of the responses to individual senses. As the dog nears and the visual and auditory cues strengthen (middle and bottom panels), the unisensory responses increase, but the relative enhancement from integration diminishes. The integration pattern shifts first to additive (combined response equals the sum) and then to subadditive (combined response is less than the sum, but still greater than the strongest single-sense response). Although all these integration types enhance detection probability compared to a single sense, the proportional benefit of combining senses is most significant when the initial cues are weakest. Adapted from (Stein & Stanford, 2008)

1.1. Neural Substrates of Multisensory Integration in the Neocortex

As discussed, multisensory integration in the cerebral cortex refers to the process by which the brain combines information from multiple sensory modalities to create a unified perceptual experience. It is a fundamental function of the cortex, particularly in cortical and cortical association areas, including the rostrolateral visual area (area RL), parietal cortex, subcortical areas like superior colliculus (SC), and multisensory regions such as the posterior superior temporal sulcus (STSp) and intraparietal sulcus (IPS) (Stein & Stanford, 2008). Studies have shown that individual neurons in these areas can respond to stimuli from different sensory inputs, often exhibiting enhanced or suppressed responses compared to unisensory stimulation, depending on the strength and alignment of the inputs (Ghazanfar & Schroeder, 2006; Stanford et al., 2005). While extensive research has focused on unisensory responses within primary and secondary sensory cortices across species, from humans to rodents (Ferezou et al., 2007; Grill-Spector & Malach, 2004; Hubel & Wiesel, 1962; Wang & Burkhalter, 2007), recent studies have uncovered sensory responses to stimuli that do not match the preferred sensory modality in regions previously thought to be strictly unisensory (Falchier et al., 2002; Iurilli et al., 2012; Laurienti et al., 2002; Molholm et al., 2002). These findings have challenged traditional views of cortical organization and prompted re-evaluating how sensory information is processed in the brain. The study by Wallace et al. provides one of the most comprehensive examinations of the distribution of the rodent cortex's uni- and multisensory responses (**Figure 2**, (Wallace et al., 2004)).

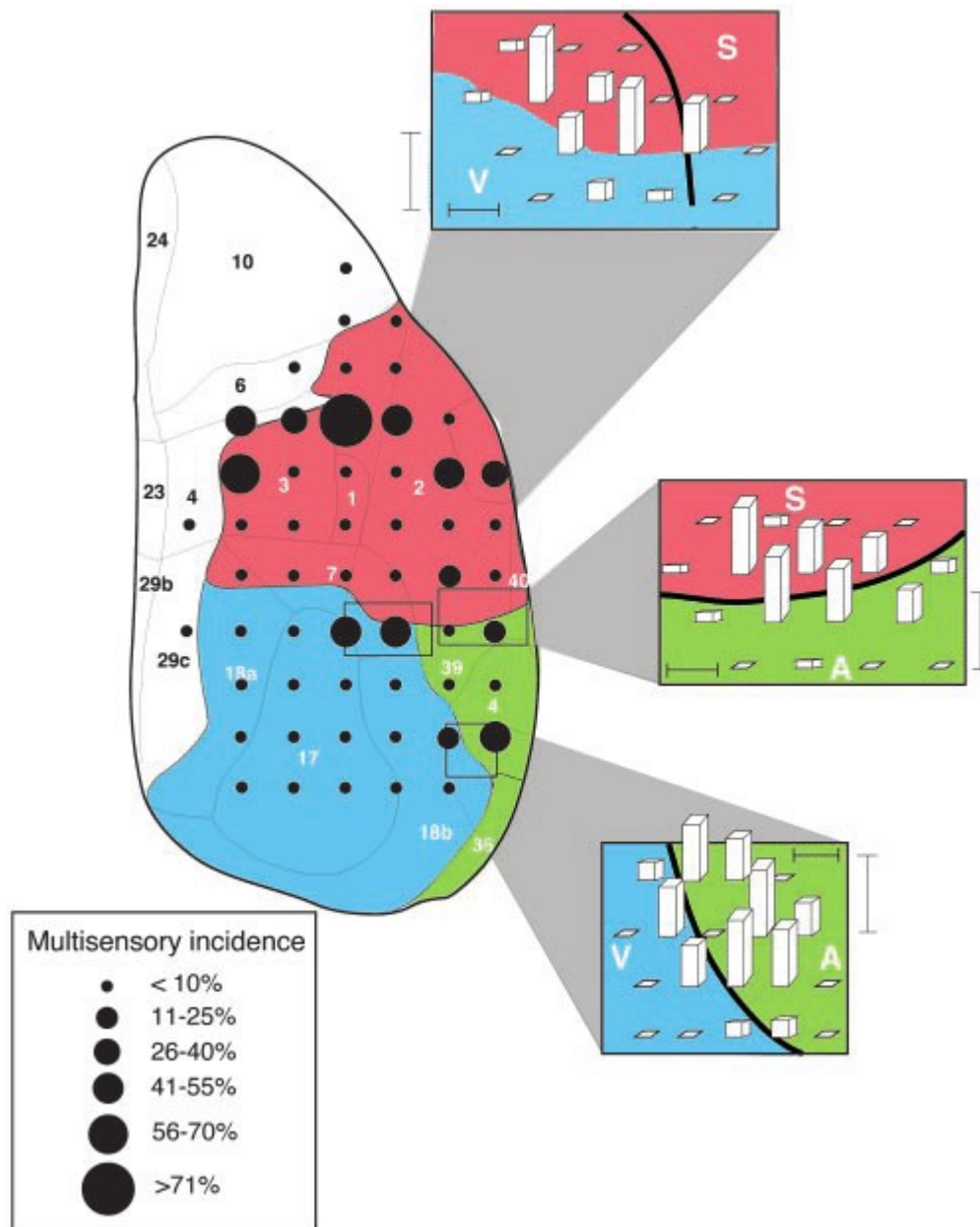


Figure 2: Distribution of multisensory neurons in the rat sensory neocortex.

The dorsal view shows major cortical areas (parietal-red, temporal-green, occipital-blue) with numbered subdivisions. Circle size at each recording site (coarse analysis in 22 rats) indicates the proportion of multisensory neurons. Insets display higher-resolution data from transition regions (9 rats), with bar height representing multisensory incidence. Abbreviations: V (visual), A (auditory), S (somatosensory) cortex. Adapted from (Wallace et al., 2004)

Overall, this research largely supported the traditional view of cortical organization, identifying predominantly unisensory responses across most of the sensory dorsal cortex in rats. However, a significant number of multisensory-responsive neurons were observed, primarily located at the boundaries between unisensory regions, particularly at the intersections of the occipital, parietal, and temporal lobes (**Figure 2**, (Wallace et al., 2004)). In rodents, these regions appear to exhibit characteristics of both secondary sensory areas and multisensory association areas (Olcese et al., 2013; Wang & Burkhalter, 2007). Notably, visual and tactile information integration in mice has been studied deeply within the intersecting area RL (Olcese et al., 2013). In this region, researchers found a mix of neurons that responded to either visual or tactile stimuli, as well as a significant proportion of neurons that responded to both modalities. A similar function was observed in the anterolateral visual area (area AL) during a study on audiovisual integration in mice (Meijer et al., 2020). This suggests that multisensory integration plays a key role in these regions in a behavioral context. A compelling example comes from Godenzini et al., who demonstrated that auditory input can significantly enhance the encoding of tactile information within the primary somatosensory cortex (S1) of mice, directly improving performance on a tactile-based goal-directed behavior (Godenzini et al., 2021).

To investigate how primary sensory cortices and higher association areas such as RL integrate multisensory information during behavior, we set out to study how activity in these regions is modulated in mice performing a behavioral task that involves the perception and discrimination of both unisensory and multisensory cues.

1.2. Multisensory Integration During Evidence Accumulation

1.2.1. Mouse as a model organism in neuroscience

Animal models are frequently employed to explore key principles in neuroscience, with the mouse becoming a predominant model organism in recent decades (Andermann et al., 2011; Carandini & Churchland, 2013). This prominence is largely due to the availability of genetic tools (O'Connor et al., 2009) and behavioral paradigms that allow for neurological measurements during active behavior (Carandini & Churchland, 2013; Mayrhofer et al., 2013). Combining complex behaviors and advanced techniques like electrophysiology and imaging makes the mouse a highly effective model system. However, like all animal models, mice cannot directly report their sensory experiences, requiring behavioral tasks to infer how they process sensory information and use it to guide behavior.

1.1.1. Behavior tasks for rodents

To investigate sensory perception in animals, obtaining a measurable output of how an individual interprets a given stimulus is essential. This is typically accomplished by having the animals engage in a behavioral task. The core objective of these tasks is to infer how sensory information is processed and translated into behavioral responses. Over the years, numerous behavioral paradigms have been developed, each optimized for the particular model organism and research question being addressed. Various rodent behavioral task designs have been reviewed extensively (Carandini & Churchland, 2013).

1.1.2. Behavioral responses as decision indicators in rodents

To assess the decision-making capabilities of animals, a behavioral readout is necessary, which can be implemented in various ways. In designs allowing free

movement, animals typically navigate to a designated location to indicate their responses and earn a reward (Scott et al., 2015). However, many advanced neurological recording techniques require the animal's head to be stabilized, leading to the implementation of head-fixed behavioral paradigms. These situations call for alternative methods of response indication. Research suggests that linking a sensory stimulus to a tracking device, which provides direct sensory-motor feedback, may facilitate quicker learning of behavioral tasks in mice (Burgess et al., 2017). Nonetheless, follow-up studies indicated that this direct sensory-motor coupling, rather than introducing a response delay, can diminish cortical signals related to response preparation, reflecting subcortical decision-making processes instead (Steinmetz et al., 2019; Zatka-Haas et al., 2021). This presents a challenge when studying cortical perceptual decision-making.

In contrast, many prior behavioral tasks involving rodents have incorporated delayed lick responses (Chen et al., 2017; Esmaili et al., 2021; Li et al., 2015; Mayrhofer et al., 2013; Musall et al., 2019). In these paradigms, a sensory stimulus is presented first, followed by a delay, after which animals can indicate their responses, effectively separating the sensory input from the behavioral output. Making it easier to study separately sensory epochs, delay (short-term memory), and decision-making. Additionally, introducing a delay period requires the cortex for bridging time gaps between sensory stimulation and choice. Because, in the case of an instantaneous coupling between input and movement, subcortical regions might suffice to achieve behavioral accuracy although the cortex might still be needed for learning. For these reasons, the behavioral task used in this thesis employed a delayed lick response for mice.

Introduction

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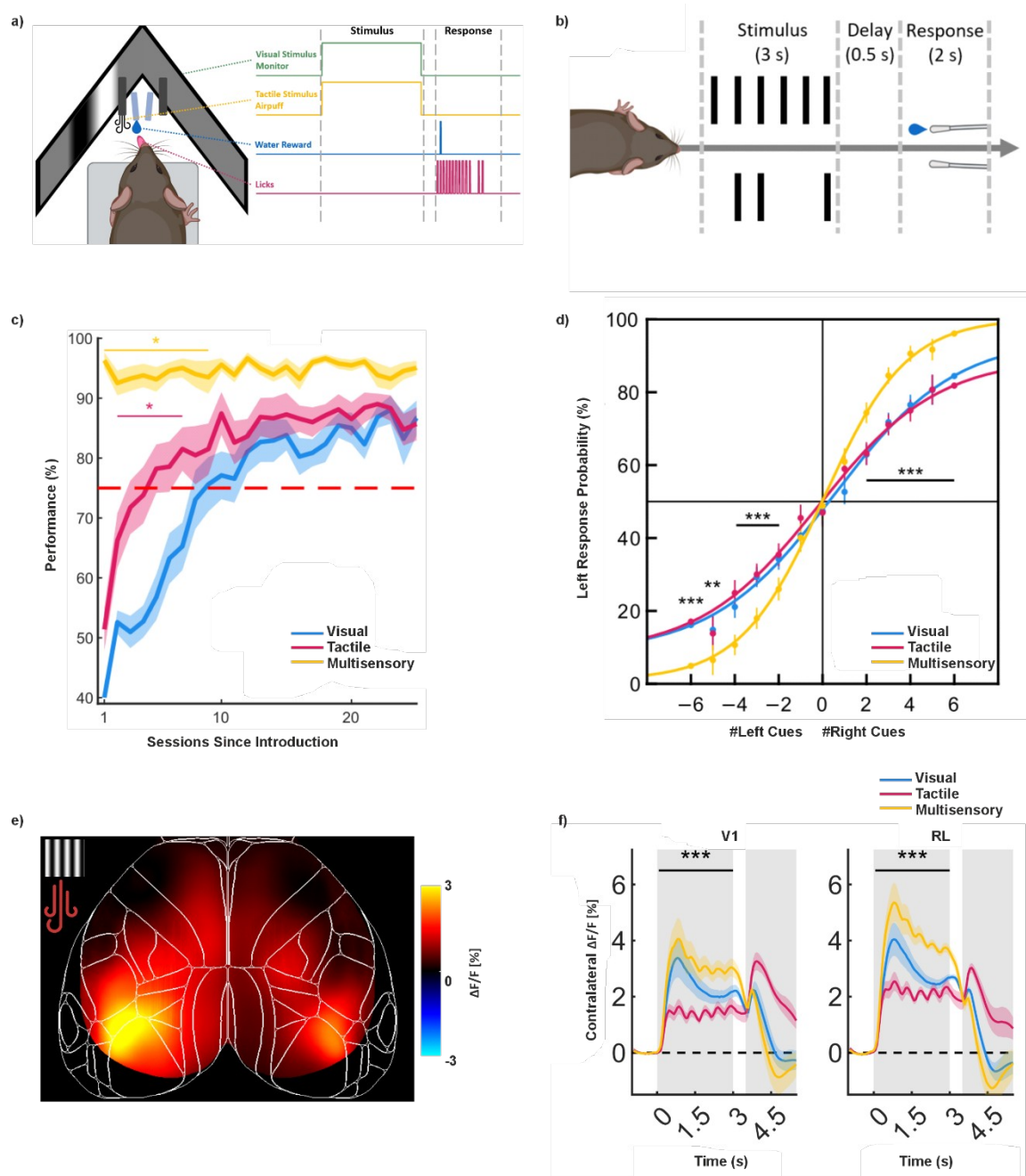


Figure 3: Behavioral Performance and Widefield Calcium Imaging During a Multisensory Evidence Accumulation Task in Mice (Nabbefeld, 2022).

- a) Schematic of the behavioral apparatus. Head-fixed, water-restricted mice were trained on a two-alternative forced-choice task. Visual stimuli (drifting gratings) were presented on monitors, and tactile stimuli (airpuffs) were delivered to whiskers. Mice reported their decision by licking one of two laterally positioned water reward spouts. Timeline indicates stimulus, delay, and response epochs.

-
- b) *Structure of the discrimination task trial. During a 3-second stimulus period, varying numbers of discrete cues were presented on the left and right sides. This was followed by a 0.5-second delay and a 2-second response window where the mouse licked the side associated with the greater number of cues for a reward.*
 - c) *Learning curves showing average performance (mean $\pm$ SEM across 5 mice) over training sessions for visual-only (blue), tactile-only (pink), and multisensory (yellow) blocks. Mice were trained sequentially on visual, then tactile, then multisensory conditions. Performance improved gradually for unisensory conditions, reaching >75% criterion (dashed red line). Multisensory performance was significantly higher from the first session.*
 - d) *Psychometric performance curves plotting the probability of a leftward choice (mean $\pm$ SEM) against the net sensory evidence (difference between # left cues and # right cues). Curves are shown for visual (blue), tactile (pink), and multisensory (yellow) trials, fitted with sigmoid functions. Multisensory performance exhibits significantly higher sensitivity (steeper slope) compared to unisensory conditions.*
 - e) *Representative widefield calcium imaging map showing average cortical activation ($\Delta F/F$ %) during task performance, likely averaged across trials or stimulus conditions highlighting task-engaged areas. Bright areas indicate increased activity, notably in posterior and lateral cortical regions, including primary sensory areas and associative regions like RL, overlaid on the Allen Mouse Brain Atlas. Icon indicates stimulus modalities potentially driving the displayed activity (visual and tactile).*
 - f) *Average contralateral $\Delta F/F$ responses (mean $\pm$ SEM) in primary visual cortex (V1, left) and rostromedial area (RL, right) during visual (blue), tactile (pink), and multisensory (yellow) trials. Shaded gray area indicates the 3-second stimulus period. In RL, the response during multisensory trials is significantly larger than the response during either unisensory condition, demonstrating multisensory enhancement. V1 primarily responds to visual and multisensory stimuli with less pronounced enhancement.*

1.2. Probing Visuotactile integration in an evidence accumulation task

To investigate the neural mechanisms underlying MSI benefits, particularly supra-linear enhancement, experimental paradigms must allow for precise control over sensory inputs and simultaneous measurement of both behavior and neural activity. Evidence accumulation tasks, where subjects integrate potentially noisy sensory information over time to make a decision, provide a powerful framework (Brunton et al., 2013; Gold & Shadlen, 2007; Pinto et al., 2019). These tasks allow researchers to parametrically vary the strength of sensory evidence and examine how neural activity reflects the integration process leading to a choice (Carandini & Churchland, 2013; Scott et al., 2015). Extending this approach to the multisensory domain enables detailed investigation into how signals from different modalities are combined within decision-making circuits (Meijer et al., 2020; Raposo et al., 2012).

The mouse model has become increasingly central to such investigations due to the availability of sophisticated genetic tools for monitoring and manipulating specific cell types, combined with the ability to train mice on complex cognitive tasks while performing chronic neural recordings (Andermann et al., 2011; Carandini & Churchland, 2013; Guo et al., 2014). Leveraging these advantages, prior research within our laboratory, done by Dr. Gerion Nabbefeld, established and characterized a robust visuotactile evidence accumulation task specifically for head-fixed mice (**Figure 3 a,b**) (Nabbefeld, 2022). In this two-alternative forced-choice (2AFC) task, head fixed mice were exposed to random sequences of visual, tactile, or multisensory cues on their left and/or right side. They were trained to report which side (left or right) received a greater number of discrete sensory cues over a 3-second interval. Visual cues consisted of grating patches, while tactile cues were brief airpuffs directed at the whiskers, ensuring modality separation. Following a brief delay, two spouts were presented to the mice, allowing them to indicate which side they perceived to have a higher number of cues. By licking the appropriate spout, they received a water reward. This task design enabled us to examine how mice integrate sensory information over time and

investigate the effects of cross-modal stimulation using Widefield Calcium imaging in awake-behaving mice.

Behavioral characterization of mice performing this task revealed significant MSI effects. While mice gradually learned the unisensory (visual-only or tactile-only) versions of the task over multiple sessions, their performance on multisensory trials (concurrent visual and tactile cues) was remarkably high (>90%) from the very first session they were introduced (**Figure 3 c**). This suggests an innate or very rapidly acquired ability to benefit from combining visuotactile information. Furthermore, psychometric analysis, relating choice probability to the net difference in sensory evidence, demonstrated significantly enhanced perceptual sensitivity during multisensory trials (**Figure 3d**). The steeper slope of the multisensory psychometric curve indicates that mice were better able to discriminate subtle differences in evidence when visual and tactile cues were combined. This behavioral enhancement aligns well with theoretical models of optimal cue integration, where combining uncertain signals improves the reliability of the overall perceptual estimate (Ernst & Banks, 2002; Fetsch et al., 2012; Gu et al., 2008).

1.3. Population-Level Neural Correlates in Parietal Association Cortex

To identify the neural basis of this behavioral enhancement, initial studies employed widefield calcium imaging across the dorsal cortex. This technique revealed broad cortical engagement during the task (**Figure 3e**) and allowed for analysis of population activity in functionally defined areas. While primary sensory cortices (V1 and S1) showed expected modality-specific responses, RL, a higher-order region situated anterior to V1 and implicated in associative processing and visuomotor integration often considered part of the broader parietal association network in mice (Harvey et al., 2012; Licata et al., 2017; Olcese et al., 2013; Wang & Burkhalter, 2007; Zhuang et al., 2017). RL emerged as a key locus for visuotactile integration. RL neurons exhibited robust responses to visual, tactile, and multisensory stimuli. Critically, the average

population activity in RL during multisensory trials was significantly greater than the response to either visual or tactile stimuli presented alone, often exceeding the linear sum (supra-additivity) (**Figure 3f**). This supra-linear enhancement was much less pronounced in V1.

The concurrence of strong behavioral enhancement with supra-linear population activity enhancement in RL strongly suggested that this cortical area plays a critical role in integrating visuotactile information to improve perceptual decisions (Fetsch et al., 2012; Stanford et al., 2005).

1.4. The Unresolved Question: Cellular and Subcellular Mechanisms of Integration

Despite successfully identifying a key behavioral phenomenon (MSI enhancement) and its population-level neural correlate (supra-linear activity in RL), these foundational studies left a critical question unanswered: how is this integration actually computed at the level of individual neurons and circuits? Widefield imaging reflects the summed activity of large, potentially heterogeneous neuronal populations, obscuring the contributions of specific cell types and their precise computations. It cannot distinguish whether the observed enhancement results from a dedicated subset of multisensory neurons, widespread gain modulation, or complex non-linear interactions within local microcircuits.

Furthermore, population imaging provides no insight into computations occurring within the dendritic trees of individual neurons. Dendrites, the branched extensions receiving synaptic inputs, are far more than passive conduits; they possess active conductances enabling sophisticated local computations, including non-linear integration of synaptic inputs (M. E. Larkum et al., 2009; London & Häusser, 2005; G. J. Stuart & Spruston, 2015). Understanding the mechanism of MSI enhancement requires investigation at this finer scale.

1.5. Dendritic Computation: A Potential Mechanism for Multisensory Integration

Layer 2/3 pyramidal neurons, the principal excitatory neurons in the superficial cortical layers, are ideally positioned to integrate diverse information streams. Their morphology features distinct input compartments: basal dendrites, located deeper in the cortex, primarily receive feedforward sensory inputs (e.g., from L4 or thalamus), while the apical tuft dendrites, extending into the uppermost layer (L1), are major targets for feedback, top-down, and long-range associative inputs (Harris & Mrsic-Flogel, 2013; M. Larkum, 2013; Olcese et al., 2013; Petreanu et al., 2009). This anatomical segregation provides a structural basis for integrating bottom-up sensory evidence with top-down contextual information or signals from other modalities converging via L1 pathways.

Crucially, dendrites are not electrically passive. They contain voltage-gated ion channels that allow them to generate local regenerative events, or dendritic spikes, such as NMDA spikes (driven by glutamate binding and depolarization) and Ca^{2+} spikes (often occurring near the apical bifurcation) (M. E. Larkum et al., 1999, 2009; L. M. Palmer et al., 2014; G. Stuart et al., 1997). These spikes enable non-linear integration, where coincident or spatially clustered inputs can evoke a much larger response than predicted by simple summation (M. E. Larkum et al., 2001; Nevian et al., 2007). Furthermore, dendrites can act as independent computational compartments, processing information locally before influencing somatic output (action potential generation) (G. J. Stuart & Spruston, 2015). Backpropagating action potentials (bAPs) from the soma can also interact with dendritic inputs, providing a mechanism for associating somatic output with synaptic activity, crucial for plasticity mechanisms like spike-timing-dependent plasticity (STDP) (Kampa et al., 2007; Magee & Johnston, 1997; Markram et al., 1997) (*Figure 4*).

Recent advances in 2Photon calcium imaging combined with genetically encoded calcium indicators (GECIs) like GCaMP have enabled direct visualization of dendritic

activity, including calcium transients associated with dendritic spikes, in awake, behaving animals (Beaulieu-Laroche et al., 2019; Chen et al., 2013; Helmchen & Denk, 2005; L. M. Palmer et al., 2014). Studies using these techniques have revealed active dendritic processing during sensory perception (N. Takahashi et al., 2016), motor learning (Cichon & Gan, 2015), reward processing (Lacefield et al., 2019), and spatial navigation (Bittner et al., 2017; Sheffield et al., 2017). Dendritic mechanisms, particularly NMDA-receptor dependent events, have also been implicated in perceptual learning and synaptic plasticity *in vivo* (Gambino et al., 2014; Gilbert & Sigman, 2007; Makino & Komiyama, 2015). These findings strongly suggest that the active, non-linear integrative properties of dendrites provide a powerful substrate for implementing the complex computations required for perception and behavior, including multisensory integration.

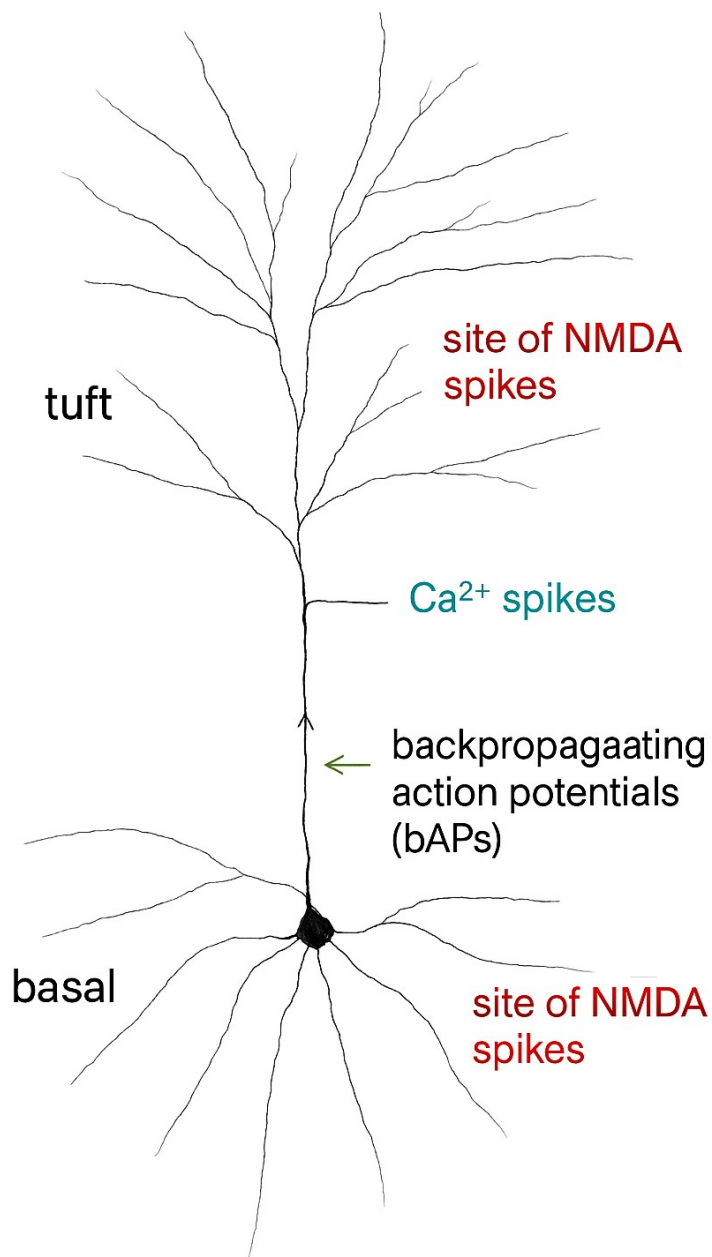


Figure 4: Schematic representation of the compartmentalization of dendritic spikes in a Layer 2/3 pyramidal neuron.

Different parts of L2/3 pyramidal neurons can generate various types of dendritic spikes according to the local distribution of conductances. Tuft and basal dendrites (top and bottom red ellipse respectively) generate local NMDA spikes. In comparison, the main bifurcation of the apical dendrite (blue region) generates Ca²⁺ spikes. The Apical dendrite (black shaft) contains Na⁺ channels for the backpropagation of action potentials (Na⁺). Adapted from (L. Palmer et al., 2012).

1.6. Perceptual Learning in the Primary Sensory Cortices

Learning is the process by which individuals acquire knowledge, skills, or behaviors through experience, study, or teaching (Lachman, 1997). Previous experiences are crucial in shaping neural activity and perception, enabling us to adapt to a constantly changing environment (LeMessurier & Feldman, 2018). As a result, both behavior and the underlying neural processes are continually adjusted by experience. These adjustments fall under Perceptual learning. This refers to the process by which sensory systems improve their ability to detect and interpret stimuli through experience and practice. This form of learning involves changes in neural processes that enable individuals to more accurately detect, categorize, or interpret sensory information (McGann, 2015). In addition, it involves functional and structural changes within the primary sensory cortices, such as V1. Studies have shown that perceptual learning can enhance sensory discrimination and improve stimulus selectivity, leading to more refined and efficient neural representations of sensory inputs (Gilbert & Sigman, 2007; Poort et al., 2015). This phenomenon is characterized by a shift in neural tuning properties and an increase in neuron's sensitivity to specific features of sensory stimuli (Crist et al., 2001; Recanzone et al., 1993). Research utilizing techniques such as functional magnetic resonance imaging (fMRI) and electrophysiological recordings has demonstrated that perceptual learning can result in long-lasting alterations in the organization and connectivity of sensory cortical maps (Schwartz et al., 2002). These changes underscore the dynamic nature of sensory processing and highlight the role of experience in shaping perceptual abilities (Pascual-Leone & Hamilton, 2001). Also, passive exposure to sensory stimuli can lead to perceptual changes in neuronal representation, which can be swiftly reversed if the stimulus gains new relevance (Fritz et al., 2005; Polley et al., 2006).

Furthermore, dendrites are proposed to play a critical role in sensory perception, serving as a pivotal site for modulating sensory processing during learning. They may provide an additional cellular mechanism for enhancing the efficiency and adaptability of

sensory representations. However, the specifics of how dendritic activity changes during learning remain an area of ongoing investigation. (Figure 5, Adapted from (Makino & Komiyama, 2015))

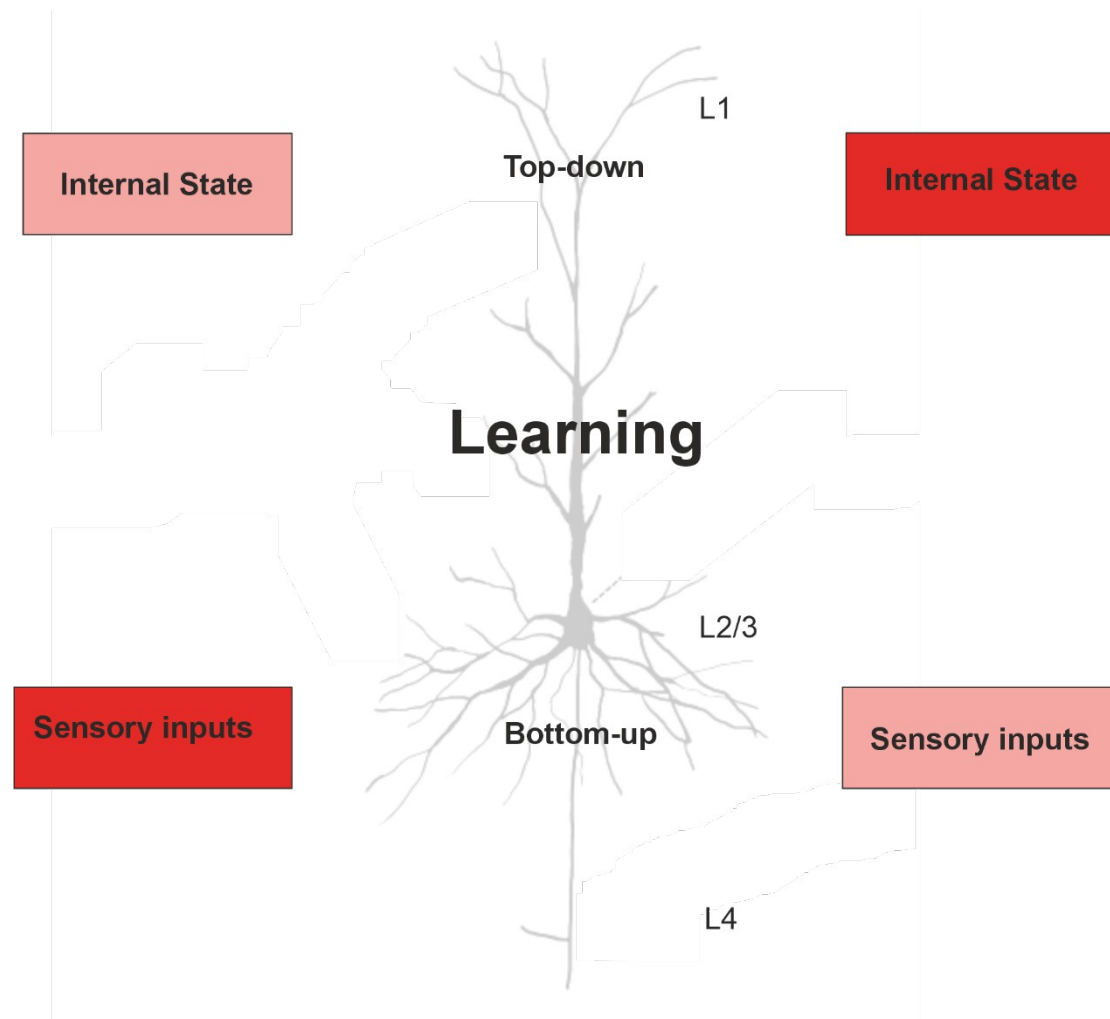


Figure 5: Dynamic changes in sensory processing occur within primary areas during learning.

In the naive state (left), neural activity predominantly arises from external sensory inputs via the feedforward (bottom-up) pathway, which primarily targets the basal dendrites of layer 2/3 (L2/3) pyramidal neurons. As learning progresses, there is an increase in feedback inputs in layer 1 (L1) and an enhanced influence of top-down modulation on sensory processing. These feedback inputs primarily interact with the distal tuft dendrites of L2/3 pyramidal neurons. Adapted from (Makino & Komiyama, 2015)

Introduction

2. Aims

Given the behavioral evidence for robust visuotactile integration in mice performing the evidence accumulation task, the population-level neural enhancement observed specifically in the parietal association area RL, and the known computational capabilities of pyramidal neuron dendrites, a crucial knowledge gap remains: **How do individual L2/3 pyramidal neurons and their dendrites within V1 and RL integrate visual and tactile information during this task to contribute to the observed supra-linear neural responses and enhanced behavioral performance?**

This thesis aims to address this central question by employing chronic 2Photon calcium imaging with cellular and subcellular resolution in awake mice performing the established visuotactile evidence accumulation task. By bridging the gap between population-level observations and single-cell/subcellular computations, this research seeks to elucidate the dendritic mechanisms underlying multisensory integration in the parietal cortex, providing crucial insights into how the brain combines information across senses to guide perception and decision-making.

2.1. Characterization of the projections from and to the rostromedial visual area

The rostromedial visual area (RL) is a higher association area previously shown to integrate visual and tactile sensory information (Olcese et al., 2013; Wallace et al., 2004). I aim to study the efferent and afferent inputs of this area of interest, as a way to reveal the circuitry involved behind the mechanism of multisensory integration. This will enable us to understand whether RL gets its information from other visual areas or is connected with subcortical areas that could influence visual processing.

2.2. Investigate the dynamics of dendritic activity while learning a visuo-tactile detection task.

Learning improves efficiency by rearranging synapses. In parallel, perceptual learning rearranges sensory representations to improve efficiency.

I aim to reveal the role of synaptic input from the primary somatosensory cortex in modulating sensory encoding in the primary visual cortex and the related behavioral output in a sensory-based task. I also investigate the changes in the encoding of sensory information in cortical dendrites in V1 and RL while learning a detection task. I addressed this by performing simultaneous 2Photon imaging of dendritic and somatic activity of L2/3 pyramidal neurons in the V1 and RL of awake-behaving mice.

We used a sparse labeling technique to perform dendritic imaging using 2Photon microscopy in vivo. To achieve sparse labeling of V1 and RL with a Ca²⁺ indicator, and to only express GCaMP8s in the cells that are transfected by Cre, a mix of 2 virus constructs is used: Cre-dependent genetic Ca²⁺ indicator GCaMP8s and diluted Cre will be injected in either L2/3.

2.3. Investigate the influence of tactile input on sensory encoding and visuo-tactile goal-directed behavior in a discrimination task.

Primary sensory cortices are widely interconnected. This facilitates the integration of multiple sensory information leading to enhanced coding efficiency during behavior. Considering that the 2-alternative forced-choice task for head-fixed mice utilizes visual and tactile stimuli, we can study sensory responses in both primary sensory cortices and association areas, such as the rostromedial visual area. With a combination of 2Photon calcium imaging and behavior training in transgenic mice (Niell-GCaMP6 mice) expressing the genetically encoded calcium-indicator GCaMP6s the neuronal activity of L2/3 pyramidal neurons will be measured. This allows us to directly record in awake behaving mice the neuronal activity mirroring the multisensory integration and enhancement seen previously (Nabbefeld, 2022).

3. Material and Methods

This section was partially adapted from (Balla et al., 2025; Nabbefeld, 2022).

3.1. Ethics

All animal procedures were performed in strict compliance with the EU directives 86/609/EEG and 2007/526/EG guidelines for animal experiments and were approved by the local government (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein Westfalen, Recklinghausen, Germany).

3.2. Animals

Data presented in this thesis was obtained from 24 female and male mice with transgenic backgrounds. 5 of these mice (2 females and 3 males) of 8-12 weeks old, expressed the genetically encoded calcium indicator GCaMP6s in the excitatory neurons of the telencephalon (Wekselblatt et al., 2016). Mice were obtained by crossing two individual mouse lines. The first mouse line (DBA-Tg(tetO-GCaMP6s)2Niell/J; JAX 024742) resulted in GCaMP6s expression under the control of tetracycline-responsive regulatory element (tetO). The second mouse line (Cg-Tg(CaMK2 α -tTA)1Mmay/DbpJ; JAX 007004) expressed the tetracycline-controlled transactivator protein (tTA) under the promoter of the forebrain-specific calcium-calmodulin-dependent kinase II (Camk2 α ; (Mayford et al., 1996)). Mice were genotyped to confirm the presence of CaMK2 α -tTA and tetO-GCaMP6s (heterozygous or homozygous). Primers used for the genotyping of tetO-GCaMP6s were 5'-GTG TCA GAG GTT TTC ACC GTC-3' (forward). Primers for CaMK2 α -tTA were 5'-GAC CTG GAT GCT GAC GAA GG-3' (forward) and 5'-GCA GCT CTA ATG CGC TGT-3' (reverse). For histological studies and the sparse labeling technique 10 female and 9 male C57BL/6J mice were included. For the sparse labeling technique 1 female and 4 male

mice were between 8-12 weeks old whereas for histology 9 female and 1 male mice were between 10-15 weeks old.

All mice were housed in fixed groups of 2-3 animals, preferably siblings. In their home cages, mice were provided with environmental enrichment and had access to food *ad libitum*. Cages were kept in a ventilated cage cabinet, which provided artificial illumination for a 12 h:12 h reversed day-/night cycle. Mice were trained during this simulated night period.

Surgeries were conducted when the mice reached an age of 8-12 weeks.

Behavioral training was started after a one- to two-week recovery from surgical procedures or after one month, allowing the viral expression. Training took place only on weekdays between 8 am and 6 pm. To motivate animals to participate in experiments, mice were kept water-restricted for the training days. Water deprivation was suspended during weekends. Throughout the water restriction, mice were monitored and scored daily. Throughout a daily training session, mice received unlimited access to water in the form of rewards for correct trial responses. If mice drank less than 1 mL during the experiment, they were later supplemented with the respective missing amount of water absorbed in oats. The stop criterion for experiments, when the weight of a mouse dropped below 20% of its reference body weight, was never reached throughout experiments.

3.3. Surgical procedure

3.3.1. Anesthesia

For viral injection, the mice were weighed and given a subcutaneous injection of Carprofen (4 mg/kg, Rimadyl, Zoetis GmbH) and Buprenorphine (0.1mg/kg, Buprenovet sine, Bayer Vital GMBH) for analgesia, 20 minutes before the surgical procedure. They were then anesthetized using (1% - 2.5%) isoflurane in oxygen and placed in a stereotaxic frame with stabilized and monitored body temperature (37°C). A moisturizing gel (Bepanthen, Bayer Vital GmbH) is applied to the eyes of the animal to prevent drying of the cornea.

1.1.1. Viral injections for histology

Following a local injection of Bupivacaine (0.08ml of 0.25% Bucain 7.5mg/ml, Puren Pharma GmbH), the skin was incised and pushed aside to reach the skull. Bilateral trephination at V1, S1, and RL is carried out at the following coordinates. (V1: antero-posterior (AP): 3 mm; lateral (L): +/- 2.5 mm and dorsoventral (DV): - 2.5 mm from the bregma) (S1: antero-posterior (AP): 1.59 mm; lateral (L): +/- 3.6 mm and dorsoventral (DV): - 2.5 mm from the bregma) (RL: antero-posterior (AP): 3.25 mm; lateral (L): +/- 2.64 mm and dorsoventral (DV): - 2.5 mm from the bregma).

Anterograde virus vectors *AAV-eGFP* and *pAAV-FLEX-tdTomato: Addgene* were injected in layer 2/3 of V1 and S1, respectively. And a retrograde virus vector was injected in layer 2/3 of RL *AAVretro-CAG-tdTomato: Addgene*.

The viruses were injected through a thin glass pipette with a micropump (UMP-3, World Precision Instruments). We injected 100 μ L at 10 nL/s per area into each mouse.

After the surgery, all animals received the same injections of Carprofen and Buprenorphine as in the beginning and were kept over a heating blanket during their immediate recovery. They also received Buprenorphine (0.009 mg/mL, Buprenovet sine, Bayer Vital GmbH) and Enrofloxacin (0.0227 mg/mL, Baytril 5%, Bayer Vital GMBH) in their drinking water for 3 days. The virus expression window was four weeks before euthanizing the mice.

1.1.2. Serial Dilution

To achieve sparse labeling of L2/3 pyramidal neurons in V1 and RL with a Ca^{2+} indicator and to only express GcaMP8s in the cells that are transfected by Cre, a mix of Cre-dependent genetic Ca^{2+} indicator GcaMP8s and diluted Cre was injected. Several dilutions of the Cre construct were tested to choose the correct one (1/6000, 1/12000, 1/15000, and 1/20000). For the Sparse labeling of the L2/3 pyramidal neurons in V1 and RL, a mix of Cre-dependent genetic Ca^{2+} indicator GcaMP8s and diluted Cre 1/15000 was injected.

1.1.3. Sparse labeling

All mice were between 8-12 weeks old. C57BL/6J male and female mice were used. A sparse labeling technique achieved dendritic imaging using 2Photon microscopy in vivo. Sparse labeling describes a method where only a small percentage of neurons will be visualized to receive a precise depiction without the disturbance of the huge number of other neurons.

1.1.4. Cranial window implantation

A 4-mm-wide circular craniotomy was then performed over the center of V1 and RL. In the C57BL/6J mice for 2Photon imaging, a combination of viral vectors (pENN.AAV.hSyn.FLEX.GCaMP8s.WPRE; Addgene, and pENN.AAV.hSyn.Cre.hGH diluted 1:15000 in phosphate-buffered saline (PBS)) was then injected through a thin glass pipette with a micropump (UMP-3, World Precision Instruments). In each mouse, we injected 50 μ L at 2 nL/s in layer 2/3 of V1 and RL (We waited around 4 weeks for stable expression before imaging). The dura was kept moist with PBS solution throughout the injection. After viral injections, a 4 mm and 5 mm wide double glass window was placed inside the craniotomy and fixed with light-curable dental cement (DE Flowable composite, Nordenta).

In the C57BL/6J mice for the 2Photon experiments, the headbar placement followed immediately after the window placement. The custom stainless steel headbar piece was held in place with quick adhesive cement (Superbond C&B, Sun Medical CO., LTD) and fast curing acrylic resin (Jet Denture repair, Lang Dental INC).

1.2. Histology and Analysis

1.2.1. Perfusion

After waiting for the injected virus to be expressed, the mice are anesthetized with an overdose of isoflurane in combination with Pentobarbital 150mg/kg; intraperitoneal injection and Buprenorphine: 0.1 mg/kg subcutaneous injection. Afterward, a phosphate buffer saline (PBS 1X) solution is infused for one minute at a rate of 5 mL/min to empty the blood vessels. The perfusion is done using Paraformaldehyde 4% (PFA) for 10 min. The brains are removed and post-fixed overnight in 4% PFA at 4°C before transferring them to a sucrose solution for at least 48h (Sucrose 20%, 4°C).

1.1.1. Slicing and Mounting

A Cryostat (CM3050 S, Leica) is used to slice frontal sections of 40 μm to recover all the brain sections. The slices are mounted sequentially on a slide to observe the projection sites. After 24 hours of drying, a coverslip is mounted on each slide in a Fluor-mount medium (Fluoromount-G with DAPI).

1.1.2. Microscopy and images acquisition

For imaging the injection sites and verifying viral expression, we acquired images using a fluorescence microscope (Invitrogen™ EVOS™ M5000 Imaging System). Imaging was performed using single z plane tile-scanning of brain slices at 1.25x. For more detailed anatomical studies, a Confocal Microscope (CMF-Zeiss LSM 980 Airyscan 2) was used. Imaging was performed using z-stack tile-scanning of brain slices, with a pinhole size of 1 A.U. (56.6 μm) and bidirectional scanning at 400 Hz. The field of view measured 581.25 $\mu\text{m} \times 581.25 \mu\text{m}$, with an image resolution of 1024 $\times$ 1024 pixels. For eGFP the excitation wavelength was 488nm and the emission wavelength was 507nm. While, for tdTomato the excitation wavelength was 554nm maximum and the emission wavelength was around 581nm. And for DAPI the excitation wavelength was 358 nm and the emission one was 461 nm.

1.1.3. Anatomical Analysis

Following that, the brain slices are aligned with the mouse brain atlas (Paxinos & Franklin, 2019). The alignment overlays the brain atlas with the microscopy pictures to know the exact brain regions seen on the slices and analyze further. Finally, the fluorescence quantification for each specific brain region was calculated and measured using a custom MATLAB script adapted from a GitHub repository from the Cortex-lab (<https://github.com/cortex-lab/allenCCF>). Additionally, FIJI (ImageJ-win64) and ARIVIS software were used for cells quantification.

1.2. Widefield Calcium imaging

1.2.1. Intrinsic imaging

To determine whether the virus injections hit the correct brain areas V1 and RL, intrinsic imaging using a widefield calcium imaging technique was used to create a phase map. The imaging was done through the cranial window (Augustinaite & Kuhn, 2021).

All recordings were aligned to the Allen CCF based on anatomical landmarks, and the location of V1 and RL was additionally confirmed using retinotopic mapping. In addition, a normal photograph of the brain through the attached window can be compared to the phase map image. By observing the visible blood vessels and comparing the locations between the two images, the specific area to image during the Multisensory Evidence Accumulation Task (MEAT) can be discovered.

1.1. Behavior

1.1.1. Visuotactile evidence accumulation task

In each behavioral session, trials involved visual, tactile, and multisensory conditions, all following a consistent framework. Each trial began with a 3-second stimulus phase, during which sensory cues were delivered to the left and/or right side of the mice. Up to six cues were presented at fixed intervals of 0.5 seconds within this period. In detection trials, up to six cues were delivered without distractors. Whereas, in discrimination trials, cues in each time-bin were determined randomly, with probabilities of 0.7 on the target side and 0.3 on the distractor side, consistent with previous research (Scott et al., 2015). To ensure the target side always had equal or more cues than the distractor side, the sequences were swapped if the distractor side had more cues by chance. Following the stimulus phase, there was a 0.5-second delay, after which a 2-second response period began. During this time, mice were presented with spouts on both sides and made decisions by licking and earning a small water reward for correct responses. A 3.5-second inter-trial interval (ITI) separated the response period from the next trial. Throughout the task, mice could run on a custom 3D-printed wheel.

1.1.1. Visual stimuli

Visual stimuli were displayed on two monitors (LG 23MB35PMF, 60 Hz refresh rate) positioned on either side of the mice. The monitors were placed in direct contact to maximize coverage of the mice's visual field, with the center of each screen aligned approximately 18 cm from the mouse's eye. The visual cues were designed to resemble a single cycle of a moving grating with a spatial frequency of 0.018 cycles per degree (cpd), covering half the monitor's width and drifting at a temporal frequency of 2 Hz to match the 0.5-second presentation period. Each cue consisted of a sinewave cycle scaled by a factor of 1.25 and clipped to a range of $[-1, 1]$, where -1 corresponded to black, 0 to gray, and 1 to white. This approach maintained the grating's overall character while slightly enhancing its contrast. The $[-1, 1]$ range was mapped into a 24-bit color

space during the presentation. The stimuli drifted from nasal to temporal on both sides of the mice, with the dark component leading and the bright component following.

1.1.2. Tactile stimuli

Tactile stimuli were delivered as discrete air puffs through stainless steel tubes (16G gavage needles, straight, 125 mm long) positioned above and in front of the mice. The tubes were angled at 45° in elevation and 10° in azimuth, directing the airflow inward toward the animals. The setup was calibrated to ensure the air puffs only deflected the whiskers near the whisker pad. Each airpuff was produced by a 20 ms digital pulse generated by a microcontroller, which activated an opto-coupled relay (AQZ105, Panasonic). This relay controlled a 24 VDC power supply (FSP-1243, 72 W, Voltcraft) that operated a solenoid valve (VDW22LA, 24 VDC, SMC). The solenoid valve regulated compressed air flow (0.03 bar), producing a brief, precise burst of airflow.

1.1.3. Water reward delivery

The microcontroller controlled the water delivery. Here, a digital pulse was generated to trigger an opto-coupled relay (AQZ105, Panasonic) switching a supply current (24 VDC, FSP-1243, 72 W, Voltcraft), which in turn opened a solenoid valve (VDW22LA, 24 VDC, SMC). The digital pulse's length controlling the individual valves' open duration was regularly calibrated to ensure a stable and equal reward size for both Valves.

1.2. 2Photon Calcium imaging

2Photon imaging was done in a custom-built setup with a resonant-scanning 2Photon microscope (CRS 8KHz, Cambridge Technology) and a femtosecond-pulsed Ti:Sapphire laser (Mai Tai DeepSee, Spectra-Physics) tuned to 920 nm. Data acquisition and controls were performed with the MATLAB-based Scanimage package. Settings and laser power were kept constant across all mice and experiments. References from

the vessel image, visual field sign maps, and/or injection site referencing were used to define the location of the regions of interest. Recordings were made from two planes covering soma in layer 2/3 of V1 at a depth of 250-300 μm below the pial surface with a frame rate of 30 Hz and their corresponding tufts dendrites in L1 at a depth of 100-150 μm below the pial surface. We used a 16x, 0.8 NA Nikon objective lens (CFI75 LWD 16X W) at a pixel resolution of 512 x 512 pixels (575 μm $\times$ 575 μm). Visual stimuli were presented at an 18 cm distance from the eye on a gamma-corrected LED-backlit LCD monitor (LG 23MB35PMF, 60 Hz refresh rate). The larger screen was used to increase the visual field coverage. Tactile stimuli were presented as short bursts of airpuffs directed towards the whiskers of the mice.

1.3. Data analysis and statistics

1.3.1. Analysis of behavior

Task performance and learning was assessed by measuring the performance of the animals in each session (number of correct trials / total number of trials).

1.1.1. Pre-processing of imaging data

The recorded data were processed using the Suite2P package (*Cortex-Lab/Suite2P*) in order to apply motion correction, neuropil extraction and ROI detection. The detected ROIs were then manually inspected. In both separated planes, to ensure the correct identification of dendrites and soma. After, the results from Suite2p and the manual curation dendrites were merged based on their activity correlation.

1.1.2. Analysis of two photon data

After pre-processing, 2Photon imaging data from each trial were aligned to the stimulus period using a custom Python script. The ROI is considered significantly responsive if it meets the following criteria Response threshold, DFOF_threshold and Trial

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consistency. Meaning, the response must exceed the baseline activity by at least 3 standard deviations. DFOF threshold should be = 0.05 (5% DFOF) so only positive responses are included. And the trial consistency which means that the ROI will be considered significantly responsive if it meets the criteria (DFOF_threshold and Response threshold) in at least 50% of the trials

Batch traces analysis processes DFOF to generate average response traces from dendritic or somatic ROIs across different stimulus conditions.

It loads metadata and data files from multiple recording sessions, filters for the relevant sessions that we are including, and extracts response traces around stimulus onset using specified parameters (e.g., baseline duration, normalization method etc.) like:

```
time_around_stimulus = 5
```

```
baseline_duration = 1
```

```
metric = 'DFOF' [metric to use (DFOF, F, spikes)]
```

```
resampling_frequency = 5
```

```
norm_type = 'diff' (Normalization type to be applied.)
```

```
sig_type = 'ranksum'
```

For each session, the code computes trial-averaged DFOF responses, statistical significance, and selects responsive ROIs based on the stimulus (like visual responders), p-values, correlation thresholds etc.

E.g., the visual plot includes only the ROIs that respond significantly to visual stimuli, regardless of whether they also respond to tactile or visuotactile stimuli.

Whereas, for dominant Visual/Tactile/Visuotactile responding ROIs, the analysis identifies ROIs that respond more strongly to one of the stimuli. For example, if the ROI is classified as dominant visual, the mean response of visual is higher than the mean response for tactile and as well as higher than the mean response to visuotactile. The plotted traces at the end extracts their responses across all conditions for comparison.

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For statistical analysis A Mann-Whitney U test was performed between the trial-averaged responses before and after stimulus onset ($p < 0.01$).

After applying all these criteria the 33 sessions that were include are divided as follow:

all sessions in RL = 12; all sessions in V1 = 21.

Passive naive (V1) = 11, Passive naive (RL) = 7, from 5 animals.

Passive trained (V1) = 6, Passive trained (RL) = 3, from 4 animals.

MS (V1) = 4; MS (RL) = 2, from 4 animals.

2. Results

1.1. Characterization of the projections from and to the rostromedial visual area

1.1.1. Retrograde tracing of areas projecting to RL

To investigate which areas project to RL, we employed a viral tracing strategy. The experimental approach, involving the targeted injection of a retrograde virus vector in layer 2/3 of RL (*AAVretro-CAG-tdTomato*), is illustrated schematically in **Figure 6a**. This strategy aimed to label neurons projecting to RL from various areas, with a particular focus on V1, S1, and associated thalamic nuclei (visual and somatosensory).

Following sufficient time for viral expression, brain tissue was processed for fluorescence microscopy to visualize the distribution of labeled projections. **Figure 6b** presents representative images from 40 μm thin coronal sections taken at different anteroposterior levels relative to bregma (AP -1.59 mm and AP -3 mm shown). These overview images confirmed the presence of robust fluorescence signals in several distal regions, including notable labeling within both V1 and S1 cortical areas, as well as within thalamic structures. Higher magnification views of these specific regions (S1, V1, and Th; **Figure 6b**, bottom row) revealed the distinction of somatic labeling, suggesting the presence of retrogradely labeled neurons in these target areas, particularly evident in the cortical regions shown.

To quantitatively assess the overall strength of projections to these areas, we measured the mean fluorescence intensity within defined regions of interest corresponding to V1, S1, the visual thalamus, and the somatosensory thalamus (**Figure 6c**). The resulting mean $\pm$ SEM fluorescence intensities were 46.27 ± 7.34 arbitrary units (a.u.) for V1 (n=3 mice), 49.42 ± 9.20 a.u. for S1 (n=3 mice), 26.41 ± 2.23 a.u. for the visual

thalamus (n=3 mice), and 33.43 ± 5.78 a.u. for the somatosensory thalamus (n=3 mice). While V1 and S1 exhibited numerically higher average intensity values compared to the thalamic regions, a one-way analysis of variance (ANOVA, $p = 0.1206$) comparing the mean intensities across all four areas revealed no statistically significant difference. Individual data points from each analyzed animal are overlaid on the bar graphs.

Given that the overall fluorescence intensity reflects contributions from both axonal projections and potential cell bodies, we next sought to specifically quantify the density of labeled somata within these regions of interest (**Figure 6d**). This analysis aimed to determine the extent of the somatic distribution of areas projecting to RL within these areas. Cell counting revealed distinct densities across the examined regions. The mean $\pm$ SEM cell density was highest in V1, measured at 82.35 ± 18.70 cells/ $10^6 \mu\text{m}^3$ (n=4 mice). S1 exhibited the second-highest density at 34.33 ± 14.82 cells/ $10^6 \mu\text{m}^3$ (n=4 mice). Considerably lower densities were found in the thalamic regions, with 15.65 ± 1.85 cells/ $10^6 \mu\text{m}^3$ in the visual thalamus and 7.11 ± 2.42 cells/ $10^6 \mu\text{m}^3$ in the somatosensory thalamus. In contrast to the overall fluorescence intensity measurements, the differences in mean cell density across these four regions were statistically significant (ANOVA, $p = 0.0136$). However, The Dunn's test with Holm correction results only revealed that the comparison between the "Thalamus Somatosensory" and "V1" regions yielded a p-value below the typical threshold of 0.05 ($p = 0.0105$), indicating a significant difference between these two regions. All other pairs of regions did not show statistically significant differences in cell density after the correction for multiple comparisons. Individual slice data points from 3 mice are shown. In addition, normality tests confirmed that most groups followed a normal distribution, except the visual Thalamus group (Shapiro-Wilk $p = 0.0063$).

Taken together, these results indicate that while the overall fluorescent signal measured from areas projecting to RL, is distributed across cortical and thalamic areas without statistically significant differences in mean intensity among V1, S1, visual thalamus, and somatosensory thalamus. However, the density of neurons labeled within these target areas varies significantly. Specifically, V1 contains the highest density of neurons

projecting to RL among the areas examined, followed by S1, with substantially lower densities observed in the visual and somatosensory thalamic regions.

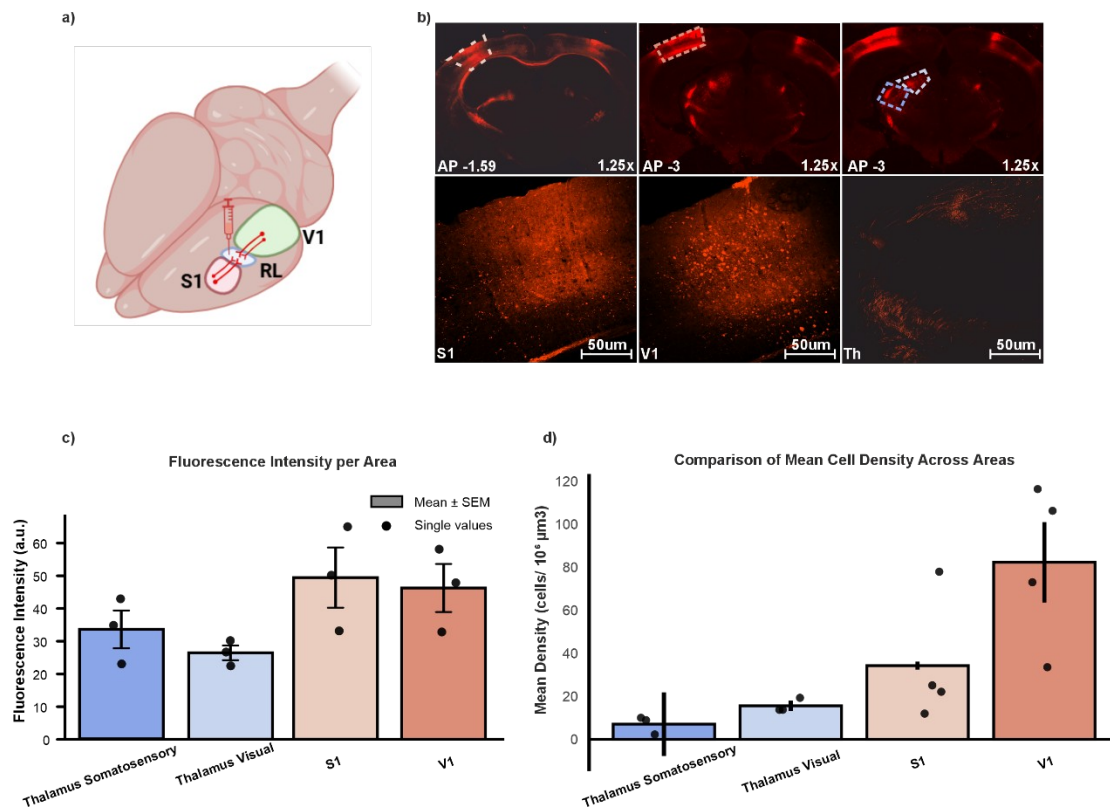


Figure 6: Characterization of projections to RL from cortical and Thalamic Nuclei.

- A schematic diagram illustrates the experimental approach, showing the location of retrograde viral vector injection in RL within the mouse brain.
- Representative fluorescence microscopy images displaying the resulting fluorescence distribution in coronal brain sections at different anteroposterior coordinates relative to bregma (AP -1.59 mm and AP -3 mm shown). Overview images (top row) demonstrate the presence of fluorescence in regions of interest like cortical areas (S1, V1) and thalamic regions (Th) indicated by dashed boxes. Higher magnification images (bottom row) provide detailed views of the fluorescence patterns within S1, V1, and the thalamus (Th), revealing cell bodies (somata).
- Quantification of the mean fluorescence intensity across the regions of interest revealed varying levels of signal. The mean $\pm$ Standard Error of the Mean (SEM), fluorescence intensity (in arbitrary units, a.u.) was measured with no significant differences across areas. (ANOVA across all areas: $p = 0.1206$). Individual data points for each animal are shown.

- d) The density of fluorescently labeled somata was quantified in the same target regions. Individual data points of imaged slices are displayed alongside the mean $\pm$ SEM. Statistical analysis revealed significant differences in mean cell density across these regions (ANOVA parametric: $p = 0.0136$). The Dunn's test with Holm correction: $p = 0.0105$). Created in BioRender. Abou Rachid, S. (2025) <https://BioRender.com/vyjbbul>

1.1.1. Anterograde tracing of areas receiving projections from V1 and S1

To map out and compare the projection targets of the primary visual cortex and the primary somatosensory cortex, we employed a dual anterograde viral tracing approach, schematically depicted in **Figure 7a**. An adeno-associated virus expressing eGFP (pAAV-eGFP) was injected into V1, while a Cre-dependent virus expressing tdTomato (pAAV-FLEX-tdTomato) was injected into S1. This strategy allowed for simultaneous labeling and differentiation of projections originating from these two distinct sensory cortices.

Successful viral expression and anterograde transport were confirmed via confocal microscopy. **Figure 7b** presents a representative 40 μ m coronal brain section at AP - 2.67 mm, illustrating the resulting fluorescence patterns. Projections labeled with eGFP originating from V1, are shown in green whereas tdTomato originating from S1 are shown in red. The projections were clearly visible in various downstream regions, including thalamic and cortical areas.

To ensure accurate anatomical localization of these projections and to ensure systematical quantification of the fluorescence intensity of these projections, acquired microscopic images were registered to the Allen Brain Atlas framework, as exemplified by the series of aligned sections shown progressing from anterior to posterior (**Figure 7c**).

We first quantified the strength of projections from V1 and S1 to several key thalamic nuclei by measuring the mean fluorescence intensity within these regions (**Figure 7d**). The mean $\pm$ SEM intensity of V1 projections (green bars) was measured as $845.46 \pm$

241.05 a.u. in the general registered thalamus region, 427.11 ± 304.92 a.u. in the Lateral Geniculate Nucleus (LGN), 448.14 ± 173.44 a.u. in the Lateral Posterior nucleus (LP), 321.63 ± 91.22 a.u. in the Ventral Posteromedial nucleus (VPM), and 412.83 ± 111.54 a.u. in the Ventral Posterolateral nucleus (VPL). For S1 projections (red bars), the corresponding intensities were 452.70 ± 97.57 a.u. (Thalamus), 292.39 ± 105.78 a.u. (LGN), 228.58 ± 65.24 a.u. (LP), 192.88 ± 69.39 a.u. (VPM), and 258.13 ± 85.97 a.u. (VPL). While V1 projections exhibited numerically higher mean intensities in most thalamic targets examined compared to S1 projections, statistical analysis using ANOVA indicated no significant difference between the projection strengths from V1 and S1 to any of these specific thalamic nuclei (Thalamus: $p=0.1619$; LGN: $p=0.6852$; LP: $p=0.2635$; VPM: $p=0.2875$; VPL: $p=0.2977$). Individual data points for each animal are displayed on the graph.

To further investigate the reciprocal projections directly between V1 and S1, quantification of the mean fluorescence intensity within distinct cortical layers of the V1 and S1 was done (**Figure 7e**). This analysis compared the strength of S1 projections terminating in V1 (red bars) versus V1 projections terminating in S1 (green bars) across layers L1 through L6b. A consistent pattern emerged across most cortical layers: projections from V1 terminating in S1 were substantially stronger than those from S1 terminating in V1. Specifically, the mean $\pm$ SEM intensity for V1 to S1 projections (green) versus S1 to V1 projections (red) were: Layer 1 (705.41 ± 169.09 vs 267.35 ± 147.35 , $p=0.0793$), Layer 2/3 (738.51 ± 169.31 vs 232.93 ± 125.30 , $p=0.0373$), Layer 4 (696.77 ± 158.72 vs 207.81 ± 111.17 , $p=0.0302$), Layer 5 (686.77 ± 173.10 vs 157.17 ± 73.09 , $p=0.0182$), Layer 6a (659.41 ± 150.06 vs 128.53 ± 52.49 , $p=0.0075$), and Layer 6b (639.65 ± 164.41 vs 122.47 ± 57.14 , $p=0.0140$). Thus, V1 projections into S1 were significantly more intense than S1 projections into V1 across layers L2/3, L4, with a trend towards significance in L1. This indicates a notable asymmetry in the strength of direct cortico-cortical connections between these two primary sensory areas asymmetric, with V1 projecting more robustly and broadly into S1 than the reverse.

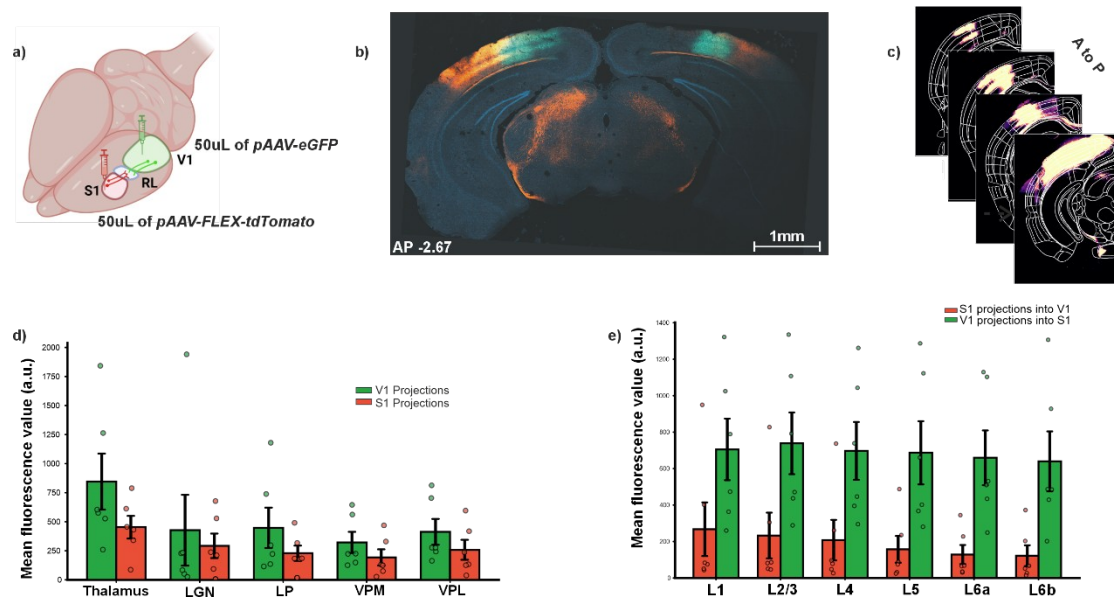


Figure 7: Dual anterograde tracing reveals distinct cortical and thalamic projection patterns of V1 and S1.

- Schematic representation of the dual anterograde viral injection strategy: pAAV-eGFP into V1 (green) and pAAV-FLEX-tdTomato into S1 (red) for anterograde labeling.
- Example confocal fluorescence images (coronal section at AP -2.67 mm) showing eGFP (green) and tdTomato (red) labeled projections.
- Example images of the alignment to the Allen Brain Atlas for anatomical registration across anteroposterior (A to P) levels.
- Quantification of mean $\pm$ SEM fluorescence intensity within specified thalamic nuclei from V1 (green) and S1 (red) projections. No significant differences were detected between V1 and S1 projections to these specific nuclei (all ANOVA $p > 0.1$).
- Quantification of layer specific mean $\pm$ SEM fluorescence intensity of reciprocal projections between V1 and S1. V1 projections to S1 (green) were significantly stronger than S1 projections to V1 (red) (ANOVA, $p < 0.05$). Individual data points are shown in (d) and (e). Created in BioRender. Abou Rachid, S. (2025) <https://BioRender.com/vyjbbul>

1.1.1. Anterograde tracing of projections from S1 and V1 into RL

Following the characterization of broad projection targets, and given the hypothesized role of RL area in integrating visual and somatosensory information, we conducted a detailed investigation into the laminar distribution of inputs arriving from V1 and S1. Anterograde tracers were injected into V1 (labeled green) and S1 (labeled red), and the resulting fluorescence intensity, representing axon terminal density, was quantified across the cortical depth of RL (see **Figure 8a** for a representative section and measurement approach). A representative slice (**Figure 8b**) illustrates these projections across all cortical layers. Quantitative analysis via paired t-tests revealed that S1 projections were significantly stronger than V1 projections in every cortical layer examined, including L1

First, we examined the average distribution patterns for each input source independently. **Figure 8c** displays the mean fluorescence intensity profile of projections from S1 (red line) plotted against cortical depth within RL (0 μm representing the pial surface). This profile reveals a distinct bimodal distribution. S1 projections show a strong presence in superficial layers, with a peak intensity around 100-200 μm depth, and a second significant peak in mid-layers, centered around 500-550 μm depth, before declining towards the deepest layers. An analysis of variance confirmed that the fluorescence intensity of S1 projections varies significantly across cortical depth (ANOVA, $p < 0.001$).

Similarly, the corresponding mean fluorescence intensity profile for projections originating from V1 (green line) terminating within RL is shown in **Figure 8d**. The laminar pattern of V1 inputs differed markedly from that of S1. V1 projections exhibited lower intensity in the most superficial layers (0-100 μm), with the primary peak of fluorescence intensity located deeper within the cortex, broadly spanning mid-to-deep layers (approximately 450-600 μm depth). A potential smaller increase was also noted near 700-750 μm . Again, the fluorescence intensity showed significant variation across cortical depth (ANOVA, $p < 0.001$).

A direct comparison of the two projection profiles is presented in **Figure 8e**, overlaying the mean intensity traces for S1 into RL (red) and V1 into RL (green) projections. And to statistically evaluate differences at various depths, the cortical depth was divided into bins (50 μm bins), and the mean fluorescence intensity was compared between the two projection sources within each bin. This analysis revealed distinct, statistically significant differences in laminar targeting between the two pathways. Projections from S1 were significantly stronger than those from V1 in the superficial cortical depths, approximately from the pial surface down to $\sim 350\ \mu\text{m}$ ($p < 0.05$, indicated by * above the red trace). Conversely, in the mid-cortical layers (roughly $375\ \mu\text{m}$ to $625\ \mu\text{m}$ depth), where V1 projections showed their highest intensity (panel d), there was no statistically significant difference in mean intensity compared to S1 projections ($p > 0.05$). In the deeper layers (below $\sim 625\ \mu\text{m}$), the mean intensities of the two projection systems appeared more comparable, with no significant differences detected with this analysis ($p > 0.05$).

Finally, to ensure that the observed differences in RL projection intensity were not merely an artifact of differential viral expression or labeling efficiency in the source areas, we quantified the mean fluorescence within the different layers of S1 and V1 themselves (**Figure 8f**). While some variability exists, this control analysis shows comparable levels of fluorescence signals within the source regions across layers, suggesting that the distinct laminar targeting patterns observed in RL (**Figure 8e**) likely reflect correct differences in projection density rather than gross disparities in initial labeling.

Collectively, these anterograde tracing results demonstrate that while both S1 and V1 project to RL, they do so with significantly different laminar specificities. S1 projections show a strong and significant preference for the superficial layers (Layers 2/3, potentially upper 4), as well as a secondary peak in mid-layers. V1 projections, while peaking in mid-to-deep layers (Layers 4-6), do not exhibit significantly stronger fluorescence intensity *compared to S1* in these layers according to our binned analysis. Directly comparing these two projections further shows a dominance of projections from S1 in L1 and upper L2/3. This confirms our hypothesis regarding S1's strong

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superficial targeting but suggests that while V1 targets deeper layers, its dominance relative to S1 is not statistically significant in those mid-to-deep zones when compared bin-by-bin. These findings underscore a specific laminar organization for the convergence of visual and somatosensory inputs within RL.

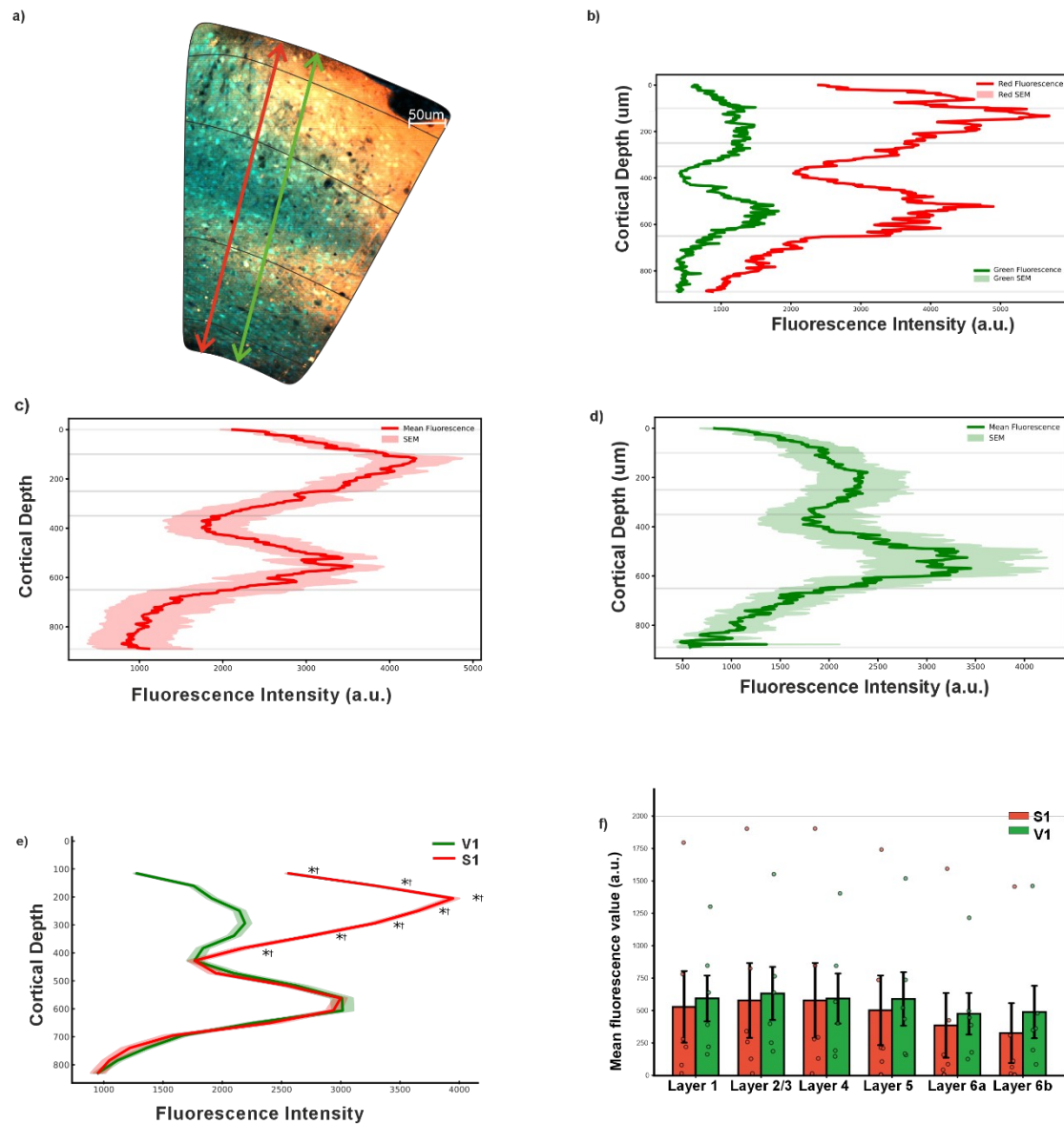


Figure 8: Laminar distribution and quantification of anterograde projections from primary somatosensory and visual cortices to RL area.

- a) *Representative confocal microscopy image section of the RL cortex illustrating the typical cortical curvature and extent. Overlaid grid lines approximate layer boundaries. Red and green lines indicate paths used for fluorescence intensity profile measurements for S1 (red) and V1 (green) projections, respectively.*
- b) *Example fluorescence intensity profiles versus cortical depth ($0\text{ }\mu\text{m}$ = pial surface) for S1 (red) and V1 (green) projections within a single RL slice, illustrating the raw data underlying the mean profiles. SEM shown as shaded areas.*
- c) *Mean fluorescence intensity profile $\pm$ SEM for all measured S1 projections across cortical depth in RL ($n=6$), showing prominent peaks in superficial and mid-layers.*
- d) *Mean fluorescence intensity profile $\pm$ SEM of all V1 projections measurements across cortical depth in RL ($n=6$), displaying a major peak in mid-to-deep layers, with lower intensity superficially*
- e) *Direct comparison of mean fluorescence intensity profiles for S1 $\rightarrow$ RL (red) and V1 $\rightarrow$ RL (green) projections, averaged across all measurements. Cortical depth was divided into $50\text{ }\mu\text{m}$ bins for statistical comparison between the two sources. Asterisks (***) indicate bins where the fluorescence intensity is significantly different between S1 and V1 projections (Paired t-test or similar appropriate test, $p < 0.0001$ – see text for full p-values per bin). S1 projections are significantly stronger in superficial layers (down to $\sim 350\text{ }\mu\text{m}$).*
- f) *Quantification of mean fluorescence intensity $\pm$ SEM within different cortical layers of the source areas (S1 - red bars; V1 - green bars). Individual data points (grey dots) represent measurements from different samples/locations. This serves as a control to assess potential differences in initial viral expression or labeling efficiency between the two injected areas, ensuring that differences observed in RL (panel e) reflect targeting patterns rather than solely expression levels.*

1.1.2. Serial dilution of a mix of viruses to optimize the sparse labeling technique

To enable 2Photon Calcium imaging of individual dendrites and soma and to achieve sparse labeling of neurons. We established a sparse labeling protocol in cortical layer

2/3. This technique involved co-injecting a Cre-dependent GCaMP virus (AAV.hSyn.FLEX.GCaMP8s.WPRE) with a second AAV expressing Cre recombinase (AAV.hSyn.Cre.hGH) that was serially diluted prior to injection, as illustrated schematically in **Figure 9a**. The aim was to titrate the Cre concentration to achieve a low probability of co-infection. The viral cocktail was targeted to layer 2/3 of the cortex to restrict expression to upper cortical layers.

We tested four different dilution factors for the Cre-expressing virus: 6,000-fold, 12,000-fold, 15,000-fold, and 20,000-fold. **Figure 9b** displays representative fluorescence images acquired from brain sections for each dilution condition. Visual inspection clearly demonstrates that increasing the dilution factor of the Cre virus resulted in progressively fewer GCaMP-expressing neurons, confirming the effectiveness of the dilution strategy in controlling labeling density. The highest dilutions (15000- and 20000-fold) achieved the most optimal labeling sparsity without compromising visibility.

To quantify the achieved sparsity, we measured the density of labeled neurons (cells/100 μm^3) using two different imaging approaches: a standard EVOS fluorescence microscope for overview analysis of fixed tissue sections, and 2Photon microscopy which allows for imaging deeper within tissue volumes, comparable to conditions used for *in vivo* calcium imaging. The results are plotted in **Figure 9c**. 2Photon calcium imaging consistently detected higher cell densities at all dilutions compared to EVOS microscopy. Using 2P imaging (dark green bars), the mean $\pm$ SEM densities were measured as 59.8 ± 00 cells/100 μm^3 for the 6,000-fold dilution, 46.9 ± 0.33 cells/100 μm^3 for 12,000-fold, 26.1 ± 2.9 cells/100 μm^3 for 15,000-fold, and 21.6 ± 2.45 cells/100 μm^3 for 20,000-fold. Data obtained using the EVOS microscope (light green bars) showed a similar trend for the dilutions where quantification was performed 12,000-fold: 32.2 ± 9.6 cells/100 μm^3 ; 15,000-fold: 18.2 ± 2.86 cells/100 μm^3 ; 20,000-fold: 15.7 ± 1.93 cells/100 μm^3 . Note that EVOS quantification was not performed for the densest 6,000-fold condition.

These quantitative results confirm that increasing virus dilution effectively reduces labeling density. Furthermore, the data suggest that the reduction in density begins to plateau at higher dilutions, as the densities achieved with 15,000-fold and 20,000-fold dilutions were relatively similar, particularly when measured with 2P imaging. Statistical comparisons (ANOVA) between the density values obtained by EVOS versus 2P imaging *within* each dilution group (12k, 15k, 20k) did not reveal significant differences (all $p > 0.1$). However, considering that 2P microscopy provides better depth penetration and volumetric coverage compared fluorescence microscopy (EVOS), the density measurements derived from 2P imaging may offer a more comprehensive assessment of the true neuronal density within the labeled volume, especially relevant for planning subsequent *in vivo* functional imaging experiments.

Based on these findings, dilutions of 1:15,000 or 1:20,000 appear suitable for achieving sparse labeling in cortical layer 2/3 using this viral combination, providing densities conducive to single-cell resolution imaging and analysis.

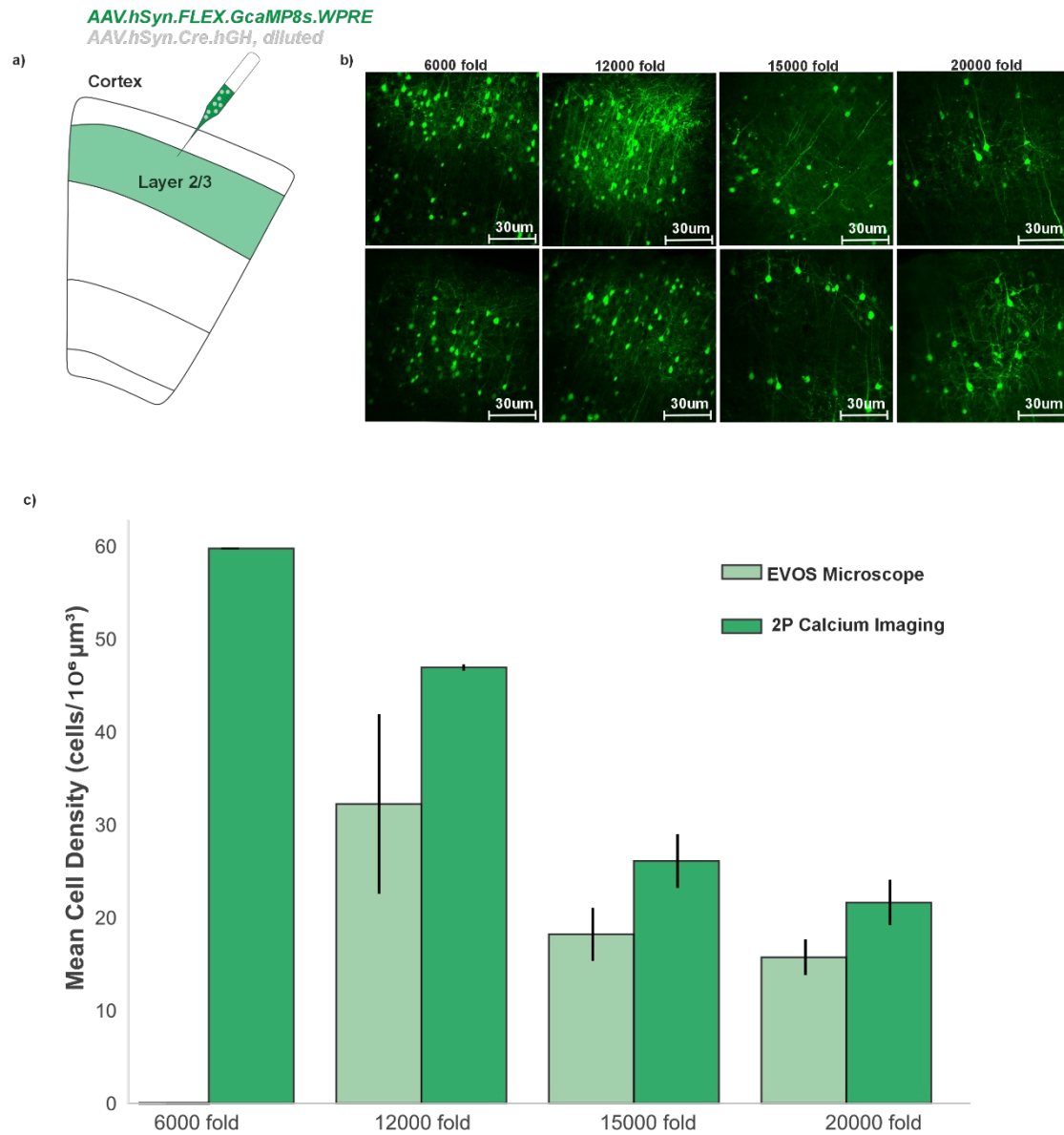


Figure 9: Optimization of sparse labeling technique in cortical layer 2/3 using viral dilution and different imaging techniques.

- a) Schematic of sparse labeling protocol: a mix of AAV1.Syn.FLEX.GCaMP8s.WPRE and diluted AAV1.Syn.Cre was injected into cortical layer 2/3 to achieve controlled expression density.
- b) Representative zoomed in images showing GCaMP-expressing neurons within L2/3 at different Cre virus dilutions (6,000 to 20,000-fold). Increased dilution leads to sparser labeling.
- c) Quantification of mean neuronal density ($\pm$ SEM, in cells/100µm³) achieved with different dilutions, measured using both EVOS microscopy (light green) and 2Photon (2P) calcium imaging (dark green). Cell density decreases with increasing dilution, reaching comparable

levels for 15,000-fold and 20,000-fold dilutions. No statistically significant difference was detected between EVOS and 2P measurements within each dilution group (ANOVA, $p > 0.05$).

1.2. Investigate the integration of visuo-tactile information in sensory discrimination task.

1.1.1. Mice learn a discrimination behavioral task

To investigate decision-making based on accumulating sensory evidence across modalities, we implemented a head-fixed behavioral task for mice detailed **Figure 10** which has previously revealed superadditive neuronal responses in the parietal area RL (see also Introduction, (Nabbefeld, 2022)). The task required water-restricted mice to report the perceived location (left or right) of the sensory stimulus to receive a water reward. **Figure 10a** illustrates the behavioral setup, featuring monitors positioned laterally for presenting visual stimuli (moving gratings) and airpuff delivery spouts directed at the whiskers for tactile stimulation. Two spouts positioned on the right and left of the mice are used for water rewards delivery. Licking behavior was monitored to record the animal's choice and reward consumption. The accompanying timeline trace shows the typical sequence within a trial, including stimulus presentation for 3s, delay period of 0.5s, choice epoch for 2s.

Mice underwent progressive training stages. Initially, they learned to associate unisensory stimuli starting with visual and introducing tactile, presented on one side (detection) with licking the reward spout on that same side. Subsequently, multisensory trials were introduced, where congruent visual and tactile stimuli were presented together on one side. The final stage involved the discrimination task (**Figure 10b**). In

this phase, stimuli were presented simultaneously on both the left and right sides during a 3-second stimulus period. The number of discrete cues (e.g., brief grating flashes or airpuffs) varied between the two sides, creating different levels of net evidence favoring either left or right. Following the stimulus presentation and a brief 0.5-second delay, the mouse had a 2-second window to report its decision by licking the corresponding side of the water spout. Trials could be visual-only, tactile-only, or multisensory (both visual and tactile cues presented concurrently, with the stronger combined evidence on one side).

We evaluated the animals' performance by plotting psychometric curves, which reflect the probability of the mouse making a leftward choice as a function of the strength of sensory evidence favoring the left side. **Figure 10c** displays the average performance data points across 5 mice for visual (blue), tactile (pink), and multisensory (yellow) conditions, plotted against the net number of cues (evidence strength). Figure 10c shows the data fitted with sigmoid functions, plotting choice probability against the numerical difference between the number of left and right cues. In **Figure 10c**, response probability increased as the cue difference grew, indicating that animals reliably chose the side with more cues. The multisensory condition (yellow) consistently resulted in higher response probabilities than either visual (blue) or tactile (pink) conditions, particularly under low cue differences (high task difficulty).

Across all three conditions, the mice demonstrated successful learning and performance of the discrimination task. The probability of choosing a particular side increased monotonically as the net sensory evidence favoring that side increased. Performance was near chance level (0.5, indicated by the dashed line) when the evidence was balanced (zero cue difference) and approached ceiling levels when the evidence strongly favored one direction representing a detection trial. Notably, visual inspection of both the raw data points and the fitted curves suggests potentially enhanced performance during multisensory trials (yellow curve) compared to unisensory trials (blue and pink curves). The multisensory curve appears steeper, particularly around the point of subjective equality (zero cue difference), and may reach higher asymptotes for stronger evidence levels, indicating that mice might integrate information across

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modalities to improve their decision accuracy, especially under challenging conditions with weak or conflicting evidence.

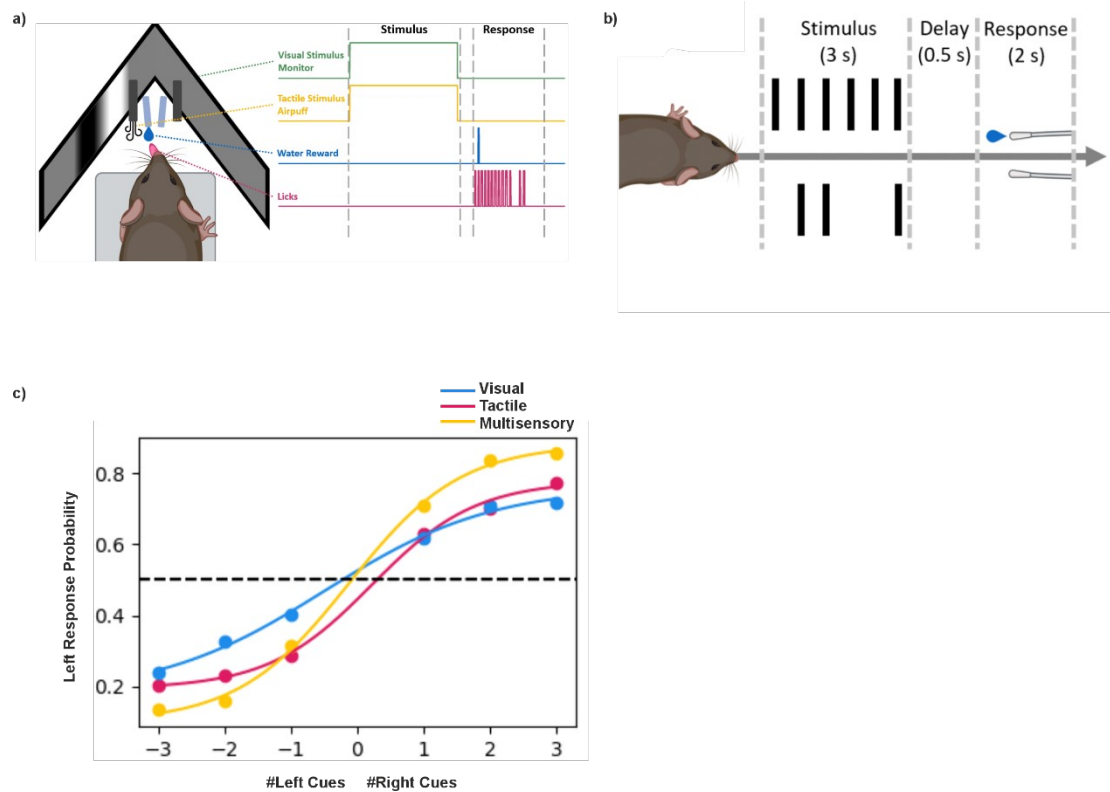


Figure 10: A multisensory evidence accumulation task for head fixed mice.

- a) Schematic representation of the behavioural detection task. Water-restricted mice are trained to respond to unisensory (visual or tactile) or multisensory (combined) cues presented randomly on either side. Visual cues are delivered as moving gratings and tactile cues as air puffs to the whiskers. A correct response is followed by water reward delivered via a lateralized lick spout.
- b) Scheme of the discrimination task. Unisensory or multisensory stimuli are presented over 3s on both sides with different number of cues, followed by a 0.5s delay and a 2s response window. The side with the greater number of cues indicates the reward location.
- c) Fitted psychometric curve showing the probability of a left response probability as a function of evidence strength for visual (blue), tactile (pink), and multisensory (yellow) trial types. Performance improved and steeper slopes in multisensory trials compared to unisensory conditions. Indicating enhanced perceptual sensitivity under multisensory conditions. Dashed line indicates chance level (0.5).

1.2. Investigate the neuronal responses to visuotactile stimuli in sensory discrimination task

1.2.1. Experimental setup and calcium imaging of cortical activity in awake mice

To investigate neuronal dynamics within specific sensory cortical areas during behavior, we employed a combination of widefield and 2Photon calcium imaging in awake, head-fixed Niell mice expressing the genetically encoded calcium indicator GCaMP6s (see Methods) (**Figure 11a**).

Initially, widefield calcium imaging was performed through a 4 mm cranial window to functionally map cortical regions responsive to visual and tactile stimuli. Using phase mapping techniques based on stimulus-evoked calcium responses across the dorsal cortex, we successfully delineated key areas of interest, including V1, S1, and RL (**Figure 11b**, left). These functionally defined regions were overlaid on vascular reference maps taken through a glass cranial window implanted after a 4 mm craniotomy, providing reliable landmarks for subsequent targeted 2Photon imaging (**Figure 11b**, right).

Following this large-scale mapping, we utilized 2Photon microscopy to achieve single cell-resolution imaging of L2/3 pyramidal neuronal activity within these functionally defined regions. **Figure 11c** shows a representative field of view obtained from Layer 2/3 within the RL area, revealing numerous GCaMP6s-expressing neuronal somata. From these data, raw calcium fluorescence signals ($\Delta F/F$) were extracted from individual neurons over the recording period (**Figure 11d**), providing traces of their activity dynamics. These traces demonstrate the feasibility of monitoring single-neuron

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activity fluctuations within functionally identified cortical areas using our experimental approach, setting the stage for analyzing neuronal population responses to specific sensory inputs or behavioral events.

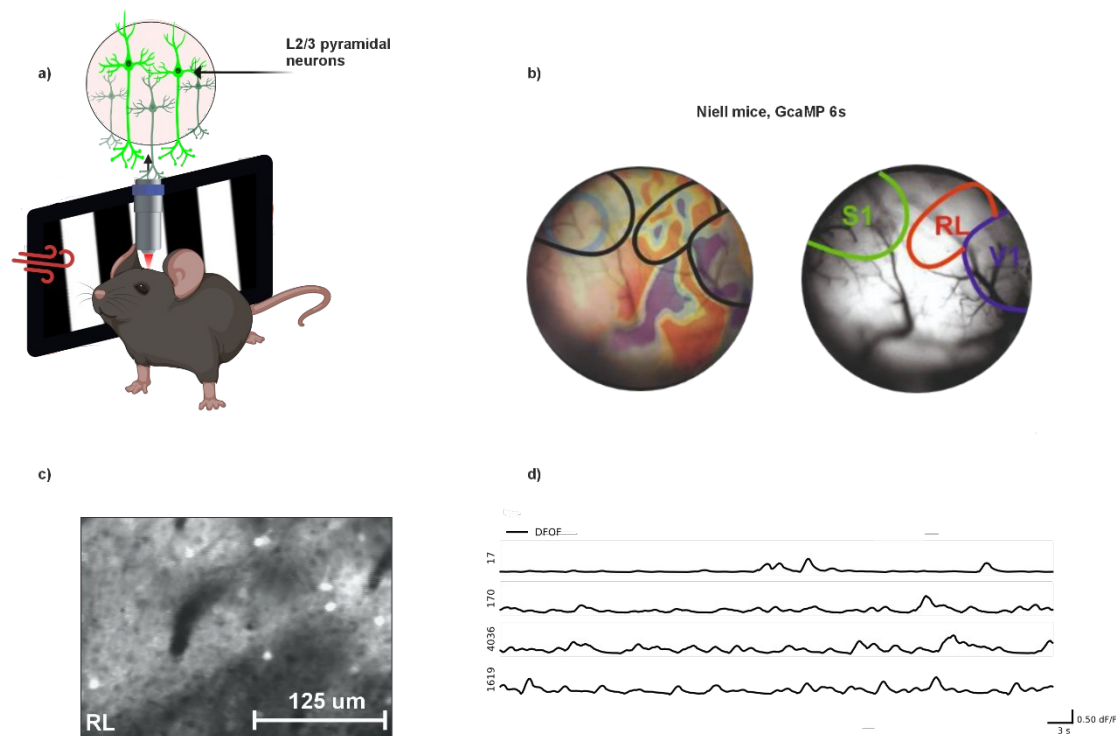


Figure 11: Experimental Setup and Calcium Imaging of Cortical Activity in Awake Mice.

- a) Schematic representation of a head-fixed mouse presented with multimodal visual (moving bar) and tactile (air puff) stimuli. Widefield calcium imaging is initially used to map cortical areas.
- b) Functional localization of cortical areas using widefield imaging in GCaMP6s transgenic mice. Left: Representative functional map obtained via widefield calcium imaging. Different colors indicate distinct cortical areas identified through phase mapping difference. Right: Overlay of functionally localized cortical areas, including V1 in blue, S1 in green), and RL in red, onto the vascular pattern visible through the 4mm cranial window.
- c) Representative field of view from 2Photon calcium imaging within the identified RL area. Bright spots correspond to the fluorescent somata of GCaMP6s-expressing neurons.
- d) Example of raw calcium fluorescence traces ($\Delta F/F$, change in fluorescence over baseline) extracted from four individual neurons within the field of view shown in (c). Traces show spontaneous and evoked neuronal activity fluctuations over time. Scale bars: vertical, 0.50 $\Delta F/F$; horizontal, 3 s. Created in BioRender. Abou Rachid, S. (2025)

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1.1.1. Neural correlates of multisensory processing and decision-making during the evidence accumulation task

To investigate the neural correlates of multisensory processing and decision-making during the evidence accumulation task, we performed 2Photon calcium imaging of neuronal activity of layer 2/3 pyramidal cells of relevant cortical areas: V1, S1, and RL. **Figure 12a** provides a schematic representation of the experimental setup, depicting simultaneous calcium imaging using ubiquitously expressed GCaMP indicators in awake mice actively engaged in the behavioral task.

We first examined the overall population activity within V1 and RL by averaging the fluorescence change (dF/F) across all recorded cells in response to different trial types (**Figure 12b**). The plots show the average neuronal response aligned to trial onset (time=0s), encompassing the stimulus presentation period (0-3s, first shaded region), a brief delay (3-3.5s, white gap), and the subsequent response/choice epoch (>3.5s, second shaded region). When pooling all responsive neurons, in V1, the population response was robustly driven by visual information; a robust increase in dF/F was observed during visual trials (blue trace). A slight stronger response was detected during multisensory (yellow trace) trials, peaking within the stimulus period. In contrast, tactile-only trials (pink trace) elicited minimal average response in V1. However, RL area exhibited distinct response properties, showing clear population activity during all three trial types. While visual and multisensory stimuli evoked the strongest responses, tactile stimuli also induced a noticeable, albeit smaller and potentially delayed, increase in average population activity. These dynamics suggest that both V1 and RL integrate multiple sensory streams.

To dissect these population responses further, we functionally classified and categorized individual neurons based on their preferential response to unisensory

stimuli or potential enhancement during multisensory conditions and analyzed their activity patterns separately (**Figure 12c**). Neurons within each recorded area (V1, RL, S1; rows) were categorized as 'Visual', 'Tactile', or 'Multisensory (MS)' preferring (columns), and their average responses (mean $dF/F \pm$ SEM) to each of the three trial types (Visual, Tactile, Multisensory) are plotted.

- In V1 (Top Row): A large population of neurons ($n=1085$) was classified as Visual-preferring. These cells responded strongly and similarly to both Visual (blue trace) and Multisensory (yellow trace) trial types, with minimal response to Tactile trials (red trace). A smaller group ($n=644$) classified as Tactile-preferring showed modest responses, primarily during Tactile and Multisensory trials. A very small number of cells ($n=19$) were classified as MS-preferring. All these cells had stimulus locked activity.
- In RL (Middle Row): The highest category of cells was classified as Visual-preferring ($n=1387$) followed by Tactile-preferring ($n=453$), as well as a notable group of MS-preferring cells ($n=48$). Visual-preferring cells responded strongly to Visual and Multisensory trials. Tactile-preferring cells responded robustly to Tactile and Multisensory trials. As expected, MS-preferring cells in RL showed increased responses during Multisensory trials compared to unisensory condition presented to that specific cell group.
- In S1 (Bottom Row): As expected, a significant population was classified as Tactile-preferring ($n=446$), responding strongly to Tactile (pink trace) followed by Multisensory (yellow trace) trials, but minimally to Visual-only trials (blue trace). A considerable number of cells were also classified as Visual-preferring ($n=374$), exhibiting responses primarily during Visual and Multisensory trials. A small group ($n=13$) was classified as MS-preferring with no differences presented for any of the stimuli.

Overall, these results reveal distinct but overlapping patterns of neuronal engagement across the recorded cortical areas. V1 and S1 exhibit strong representation primarily for their preferred modality, although cross-modal responses are also present. RL, on the other hand, displays broader tuning, with large populations responsive to visual, tactile,

and multisensory stimuli, suggesting a role in integrating information across modalities during the decision-making process. The presence of MS-classified cells, particularly in RL, further supports the potential for specialized multisensory integration mechanisms within this network. Even though, the mechanism of the multisensory enhancement seen in RL as a multimodal integration hub, is still unclear.

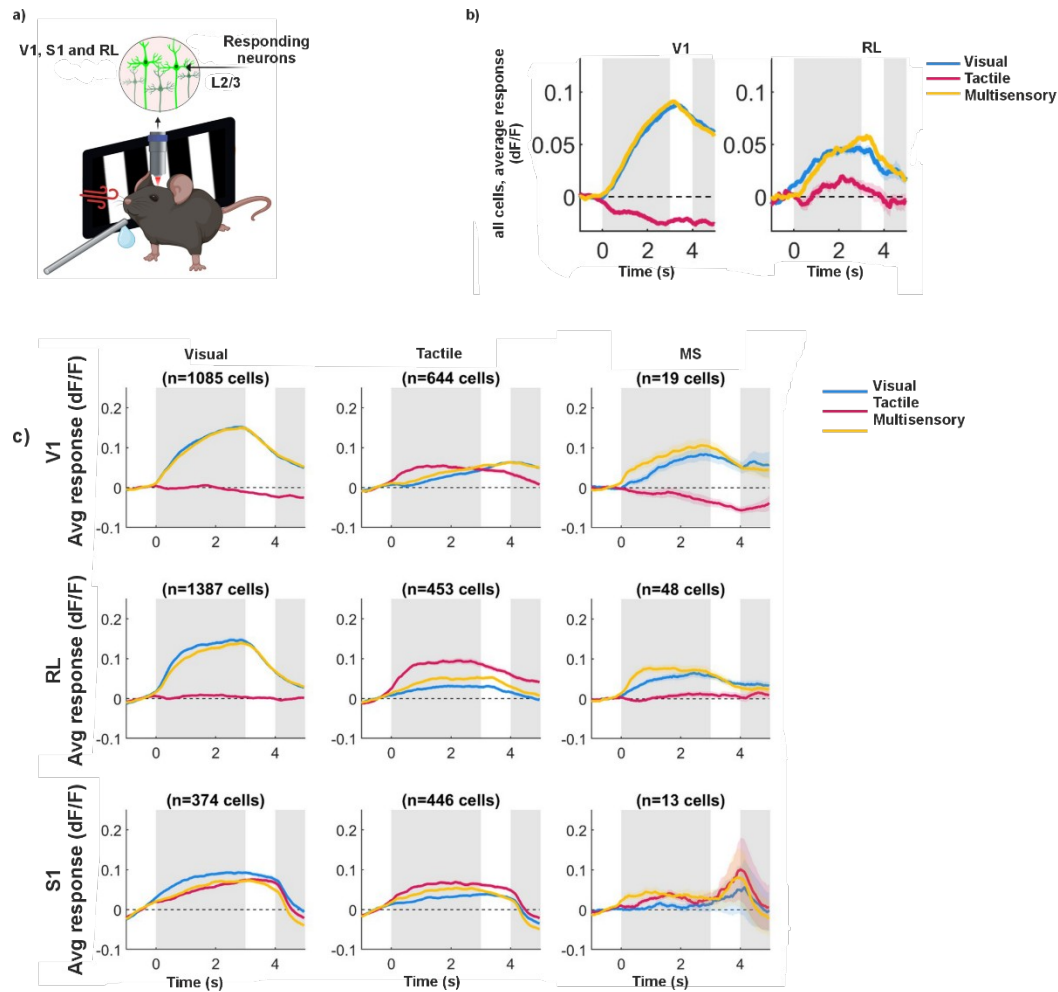


Figure 12: L2/3 pyramidal neuronal calcium activity in V1, RL, and S1 during the multisensory evidence accumulation task.

- a) Schematic illustrating simultaneous 2Photon calcium imaging of L2/3 neurons in V1, S1 and RL during a behavioral task. Imaging was performed in awake, behaving mice during task engagement.

- b) Average population response (mean $\pm$ SEM, dF/F %) across all recorded cells in V1 (left) and RL (right) during Visual (blue), Tactile (pink), and Multisensory (yellow) trials. Shaded areas indicate stimulus (0-3s) and response (>3.5s) periods. V1 shows strong visual/multisensory responses; RL responds to all modalities.
- c) Average response traces (mean $\pm$ SEM, dF/F %) for functionally defined neuronal populations within V1, RL, and S1 (rows). Columns separate cells based on modality specificity responsiveness to Visual, Tactile, or Multisensory stimuli. Traces show responses of these specific cell groups during visual, tactile, and multisensory trial types. Cell counts (n) are indicated for each subgroup. Modality-specific tuning is evident, particularly in V1 and S1, while RL shows more mixed selectivity. Created in BioRender. Abou Rachid, S. (2025) <https://BioRender.com/x0d4c44>

1.1. Investigate the dynamics of dendritic integration of visual and tactile sensory inputs while learning a visuo-tactile detection task.

1.1.1. Experimental setup of simultaneous calcium imaging of somatic and dendritic activity in V1 and RL

To investigate how individual neurons in layer 2/3 of associative and primary visual cortices integrate multisensory information, we established an experimental paradigm combining sparse neuronal labeling with chronic 2Photon calcium imaging in awake mice. We targeted V1 and RL in C57BL mice via localized injections of a cocktail of virus including cre and cre dependent AAV carrying the GCaMP8s calcium indicator. A cranial window was implanted over the dorsal cortex to provide optical access for both functional mapping and 2Photon imaging (**Figure 13 a,b**). This strategy resulted in

sparse labeling of L2/3 neurons, allowing for clear visualization of individual neuronal morphology, including extensive apical dendritic arbors, as confirmed by volumetric imaging (**Figure 13d**).

To localize regions of interest for 2Photon recording, we first conducted intrinsic signal imaging through the cranial window. Mice were exposed to visual stimuli (drifting bar), and functional activation maps were obtained, revealing strong stimulus-evoked signals in areas V1 and RL (**Figure 13c**, top). These signals were overlaid on the vascular image to identify precise cortical coordinates for imaging and to confirm alignment of the injection sites (**Figure 13c**, bottom). This procedure was critical as the mice did not express in the whole cortex calcium indicator, requiring localized GCaMP8s expression to target our imaging planes. A side view reconstruction of the injection site revealed vertically aligned dendritic arbors extending across cortical layers (**Figure 13d**), providing insight into the morphological characteristics of the labeled neurons.

Using the 2Photon microscope during head-fixation, we then achieved simultaneous calcium imaging from neuronal somata and their corresponding apical dendrites within the sparsely labeled population by acquiring data from distinct focal planes (**Figure 13e**, left panel). Analysis of the raw fluorescence signals ($\Delta F/F$) revealed distinct calcium transients in both somatic and dendritic compartments (**Figure 13e**, right panel). These transients were frequently observed in temporal proximity to the presentation of various unimodal and multimodal sensory stimuli (visual, auditory, tactile), demonstrating the successful recording of compartmental neuronal activity dynamics within identified cortical regions V1 and RL in behaving mice.

These results suggest that both dendrites and somata of L2/3 neurons in RL and V1 exhibit differential activity patterns depending on the sensory modality, offering insight into how multisensory signals are processed at the subcellular level.

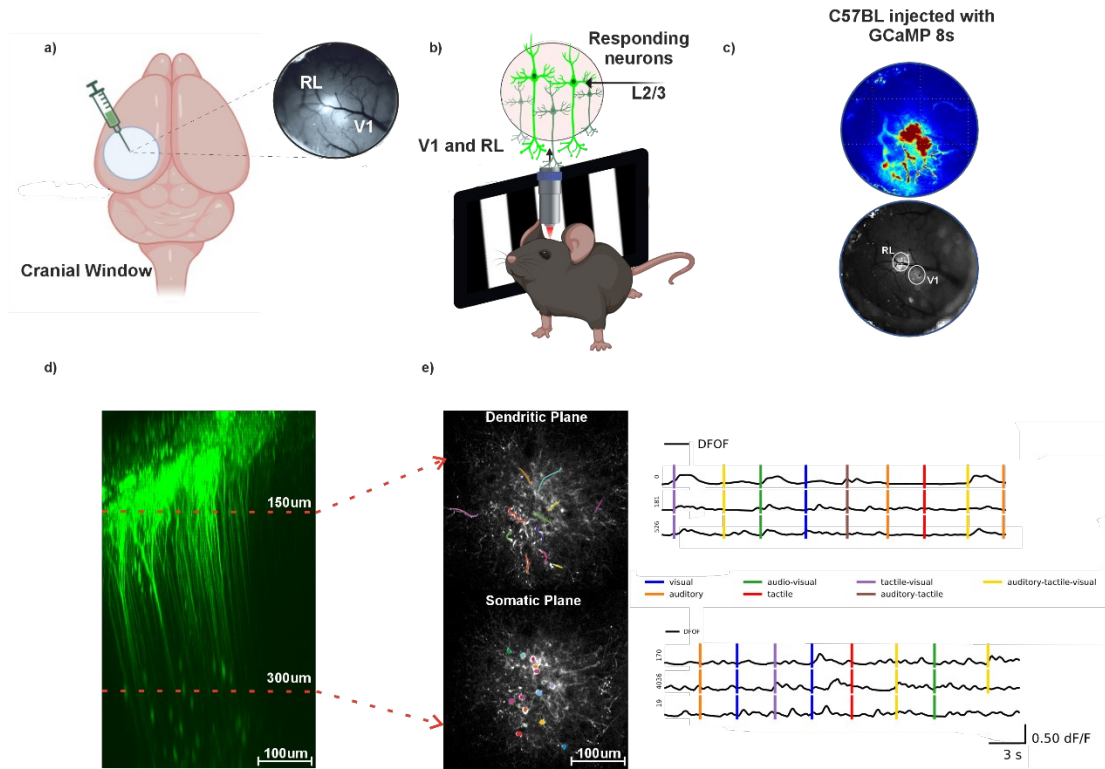


Figure 13: Sparse Labeling and Calcium Imaging of Somatic and Dendritic Activity in V1 and RL.

- Schematic illustration of the surgical procedure involving targeted injection of AAV-GCaMP8s for sparse labeling of Layer 2/3 (L2/3) pyramidal neurons in V1 and RL and implantation of a chronic cranial window covering the dorsal surface of the cortex in C57BL mice.
- Experimental setup for intrinsic imaging of head-fixed mouse presented with visual stimuli (drifting bar). with simultaneous widefield calcium imaging to localize functionally responsive cortical regions.
- Intrinsic signal imaging used to functionally identify cortical areas RL and V1 in C57BL/6 mice injected with GCaMP8s. Top: functional activity heatmap following visual stimulation, in a C57BL mouse injected with GCaMP8s. Bottom: injection sites mapped onto the vascular image, confirming coverage of RL and V1 indicated by white circles.
- Side-view of maximum intensity projection reconstructed from a volumetric 2Photon scan of a sparsely labeled region, demonstrating the successful expression of GCaMP8s in L2/3 pyramidal neurons and visualization of their apical dendrites. Dashed lines indicate approximate depths of planes shown in (e) dendritic plane at 150μm and somatic plane at 300 μm from the pial surface.

e) *Simultaneous 2Photon calcium imaging of dendritic and somatic compartments. Left: Representative fields of view at a superficial dendritic plane (top) and a deeper somatic plane (bottom) within the targeted cortical area. Colored overlays indicate example regions of interest (ROIs) corresponding to dendritic segments and neuronal somata. Right: Example raw calcium fluorescence traces ($\Delta F/F$) from selected dendritic (top) and somatic (bottom) ROIs shown on the left. Vertical colored lines indicate the timing of different sensory stimulus presentations (visual, auditory, tactile, and combinations thereof, see legend). Scale bars: vertical, 0.50 $\Delta F/F$; horizontal, 3 s. Created in BioRender. Abou Rachid, S. (2025) <https://BioRender.com/x0d4c44>*

1.1.1. Mice learn a detection task while simultaneously recording dendritic and somatic plane

To investigate the neural mechanisms underlying decision-making based on accumulating evidence across different sensory modalities, we developed and implemented a head-fixed, two-alternative forced-choice (2AFC) behavioral task for mice (**Figure 14**). This paradigm allowed for precise stimulus control and compatibility with in vivo physiological recordings.

As illustrated in **Figure 14a**, water-restricted mice were head-fixed in the behavioral apparatus. This setup featured two computer monitors positioned laterally to present visual stimuli (e.g., moving gratings) independently to the left or right visual hemifield. Tactile stimuli were delivered via precisely controlled airpuffs directed at the whiskers. Two lick spouts, positioned symmetrically on the left and right, served both for delivering water rewards. The typical trial structure, depicted in the timeline trace, involved a stimulus presentation period (3 seconds), followed by a short delay (0.5 seconds), and then a response window (2 seconds) during which the mouse had to lick the spout corresponding to the side of stimulus presentation to receive a water reward.

Mice underwent a structured, multi-stage training protocol to learn the task contingencies. Initially, they were trained exclusively on the visual modality

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(unisensory visual trials). They learned to associate a visual stimulus presented on one side (e.g., left) with licking the corresponding spout (left spout) to obtain a reward. Once an individual mouse achieved a consistent performance level of at least 75% correct trials on the visual task (indicated by the dashed red line in **Figure 14 b,c**), unisensory tactile trials were introduced. In these trials, mice learned the analogous association for the tactile stimulus. After demonstrating proficiency in both unisensory tasks individually, multisensory trials were introduced, where congruent visual and tactile stimuli were presented simultaneously on the same side.

Figure 14b displays the average learning curves for a cohort of mice ($n=5$), plotting the performance (fraction of correct trials, mean $\pm$ SEM) against the number of training sessions since the introduction of each specific stimulus type. As shown by the blue (Visual) and pink (Tactile) curves, mice typically required multiple training sessions (ranging from approximately 5 to 15 sessions, with inter-animal variability) to reliably perform above the 75% criterion for both unisensory conditions. This indicates a gradual learning process for associating each unisensory cue with the correct motor response.

A key observation, highlighted by the yellow curve, is the performance on the multisensory trials. When these trials were introduced after the animals had learned the unisensory tasks, performance was immediately high, typically exceeding 90% correct from the very first session containing multisensory stimuli. This suggests that extensive learning was not required for the multisensory condition itself; rather, the mice appeared to effectively integrate or combine the information from the already-learned unisensory cues.

Figure 14c shows exponential functions fitted to the learning data for individual mice (faint lines) and the population average (bold lines). These fitted curves provide a clearer visualization of the learning dynamics, emphasizing the slower acquisition rates for the visual and tactile tasks compared to the rapid attainment of high performance in the multisensory condition.

Together, these findings indicate that while unisensory trials require training and individual adaptation, multisensory integration allows for immediate and high-performing task execution, underscoring the computational advantages of multisensory processing in behaviorally relevant contexts.

To track the emergence and refinement of sensory representations and experience and to investigate decision-related activity during learning, we employed a longitudinal experimental design incorporating both passive stimulus exposure and active behavioral training, while simultaneously monitoring neuronal activity in relevant cortical areas (**Figure 14d**). The experiment commenced with characterizing baseline stimulus-driven neuronal responses in naive animals in the absence of task context (Passive Naive phase). During this initial phase, mice were head-fixed but not water-restricted and were passively exposed to a battery of sensory stimuli. These stimuli included unisensory presentations (visual moving gratings, tactile airpuffs to the whiskers, auditory tones/noise bursts) delivered to either the left or right side, as well as various multisensory combinations (visual-tactile, visual-auditory, tactile-auditory, and visual-tactile-auditory). Importantly, no behavioral response was required, and no reward was delivered. Throughout this and all subsequent phases, we performed *in vivo* 2Photon calcium imaging of the soma and dendrites of layer 2/3 pyramidal neurons in V1 and RL, areas implicated in visual and tactile/multisensory processing, respectively.

Following the naive passive characterization, mice were placed on a water restriction schedule and began Visual Training in the active 2AFC discrimination task, learning to associate the visual stimulus location with the correct lick response for a water reward. Once mice achieved proficient performance on the visual task (e.g., >75% correct consistently), but before the introduction of the tactile stimulus, they underwent another Passive Stimulation session, identical to the naive session, allowing comparison comparison point to assess how training, learning and reward context modulate sensory encoding. Subsequently, Tactile stimuli were introduced into the active training paradigm, requiring mice to learn the tactile stimulus-response association (often interleaved with visual trials). After reaching proficiency in the tactile task, a further Passive Stimulation session was conducted. Finally, congruent multisensory (visual-

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tactile) trials were introduced (MS Training), leveraging the previously learned unisensory associations. Once mice demonstrated expert performance across all conditions (visual, tactile, and multisensory), a final Passive Trained session was performed to assess sensory representations in the fully trained state.

This longitudinal approach, combining interleaved passive recordings with stages of active learning, enabled us to dissect how neuronal representations in V1 and RL evolve due to passive experience versus active, goal-directed learning of unisensory cues and their subsequent integration in a multisensory context.

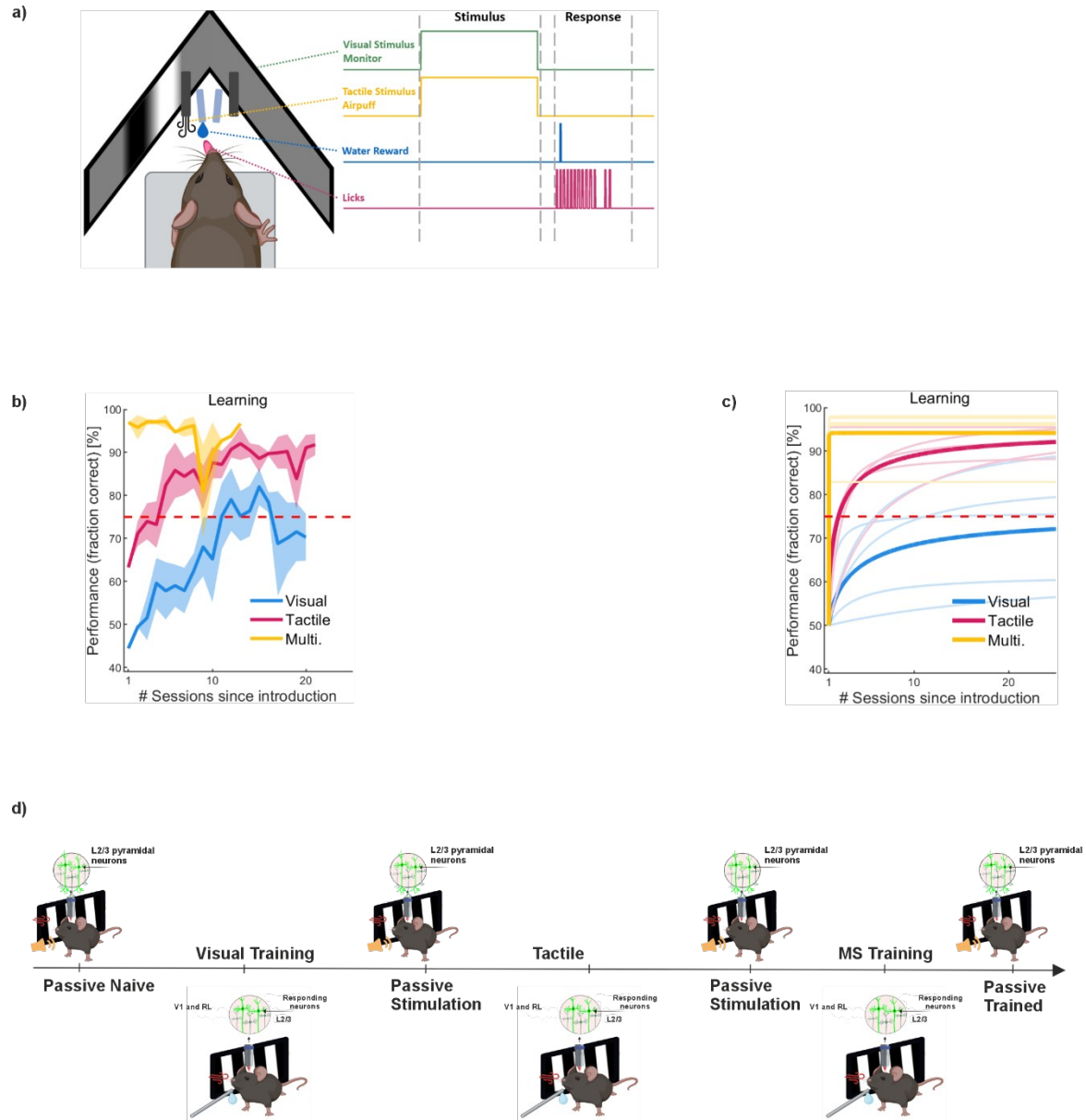


Figure 14: Multisensory evidence accumulation task during simultaneous 2Photon calcium imaging of dendritic and somatic plane.

- a) Left: Schematic of the head-fixed behavioral apparatus. Mice are presented with visual stimuli (moving gratings) on lateral monitors or tactile stimuli (airpuffs) via spouts directed at the whiskers. Mice report the perceived stimulus location (left or right) by licking the corresponding water reward spout. Right: Timeline illustrating a typical trial structure, including stimulus presentation (3s), a delay period (0.5s), and a response/choice window (2s) where licks are recorded and reward is delivered upon a correct choice.

- b) *Average learning curves (mean $\pm$ SEM across $N=5$ mice) showing performance (fraction correct trials) over training sessions since the introduction of each stimulus type: visual (blue), tactile (pink), and multisensory (yellow). Mice were trained sequentially, starting with visual, then tactile (introduced after reaching 75% criterion on visual), followed by multisensory trials. The dashed red line indicates the 75% performance criterion (chance level).*
- c) *Fitted learning curves (exponential fits) for individual mice (faint lines) and the population average (bold lines) for the same conditions as in (b), illustrating the differential learning rates and asymptotic performance levels.*
- d) *Longitudinal experimental design combining passive sensory stimulation and active behavioral training with 2Photon calcium imaging of L2/3 pyramidal neurons in RL and V1. Timeline illustrating the sequence of experimental phases. Created in BioRender. Abou Rachid, S. (2025) <https://BioRender.com/x0d4c44>*

4.4.3. Neural classification based on the amplitude of responses

To systematically identify neurons significantly modulated by sensory stimuli, we analyzed the somatic calcium transients (dF/F) from individual ROIs on a trial-by-trial basis. **Figure 15** provides representative examples of dF/F traces for different stimulus conditions, illustrating the response patterns and the application of our responsiveness criteria. An ROI was classified as significantly responsive to a specific stimulus condition only if it met a stringent set of four criteria. Firstly, the mean post-stimulus response (averaged across all trials for that condition) had to be statistically significantly greater than the mean pre-stimulus baseline activity, assessed using a one-sided Mann-Whitney U test (ranksums function, alternative='greater'). Secondly, to ensure the evoked response was distinguishable from background fluctuations, the peak or mean post-stimulus response was required to exceed the baseline activity (defined as the activity in the 1-second window before stimulus onset) by at least three times the standard deviation of that baseline period. Thirdly, only positive deflections indicating an increase in fluorescence were considered, with a minimum required peak or mean

amplitude of 0.05 dF/F (5% change). Finally, to ensure the response was reliable and not driven by outlier trials, the ROI had to meet the amplitude-based criteria (both the 3*SD threshold and the 0.05 dF/F threshold) in at least 50% of the individual trials presented for that specific stimulus condition.

Figure 15 showcases examples of this classification. For instance, the ROI shown in **Figure 15a** exhibited a clear, statistically significant increase in mean dF/F following contralateral visual stimulation ($p = 1.9e-5$), with numerous individual trials (light green lines) surpassing the amplitude thresholds, leading to its classification as responsive to this stimulus. Conversely, the same ROI (**Figure 15b**), despite being subjected to the same visual stimulus type but on the ipsilateral side, showed a flat mean response ($p = 0.44$) and few individual trials exceeding threshold (grey lines dominate), thus failing the responsiveness criteria for this condition. Similarly, **Figure 15c** shows a non-significant response to contralateral tactile stimuli ($p=0.30$). In contrast, the ROI depicted in **Figure 15 d, e, and f** demonstrates significant mean responses ($p=0.013$, $p=0.001$, $p=0.018$ respectively) to ipsilateral tactile, contralateral visual-tactile, and ipsilateral visual-tactile stimuli, accompanied by visible trial-level activity, illustrating cases likely meeting the full set of criteria. This multi-faceted approach ensured a robust identification of ROIs with genuine, stimulus-locked activity rising above noise and trial variability.

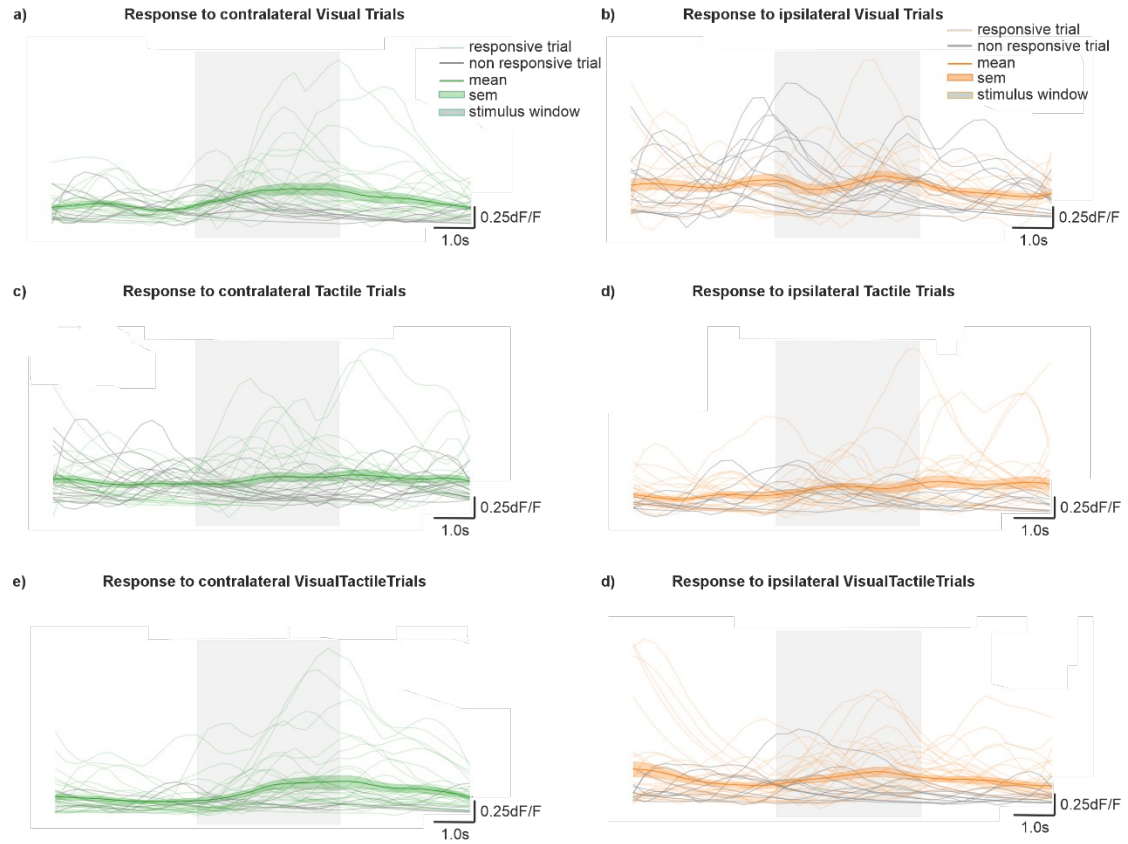


Figure 15: Example somatic calcium traces illustrating criteria for responsiveness classification.

Representative dF/F traces from individual neuronal somas showing responses to different sensory trial types.

- a) Responses to contralateral visual stimuli.
- b) Responses to ipsilateral visual stimuli
- c) Response to contralateral tactile stimuli.
- d) Response to ipsilateral tactile stimuli.
- e) Response to contralateral visual-tactile stimuli.
- f) Response to ipsilateral visual-tactile stimuli.

In each panel, thin lines represent individual responsive trials (light green/orange) or non-responsive (grey) based on amplitude thresholds (see Methods Chapter). The thick line indicates the mean dF/F response across all trials, with the shaded area representing the standard error of the mean (SEM). The grey shaded box indicates the stimulus presentation window (3 s). Scale bars: horizontal = 1.0 s, vertical = 0.25 dF/F.

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These examples illustrate the data used to determine significant ROI responsiveness based on statistical significance (Mann-Whitney *U* test comparing pre- vs. post-stimulus mean response, *p*-values shown), response magnitude relative to baseline noise, absolute dF/F change, and trial consistency. *P*-values for the Mann-Whitney *U* test on the mean traces shown are: (a) $p = 1.9e-5$, (b) $p = 0.44$, (c) $p = 0.30$, (d) $p = 0.013$, (e) $p = 0.001$, (f) $p = 0.018$.

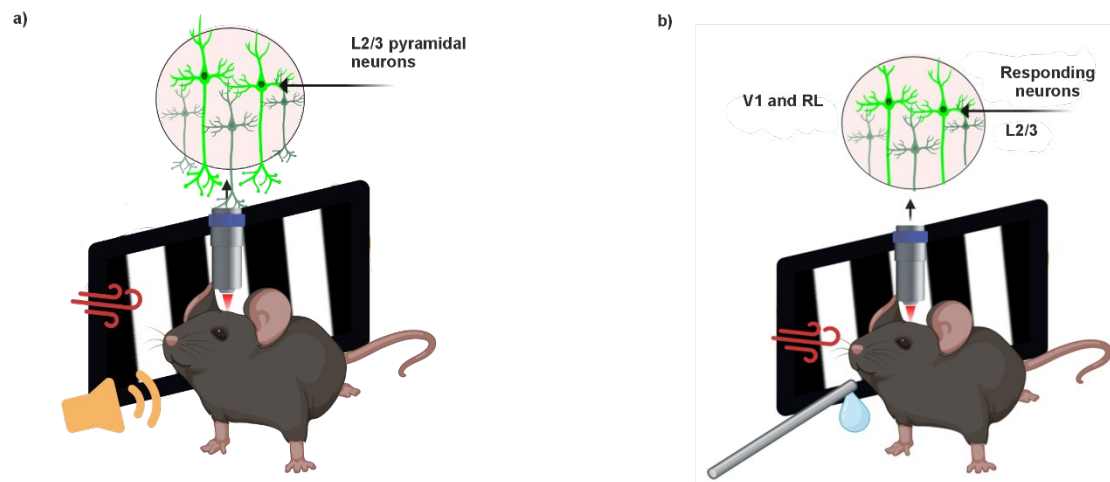


Figure 16: Experimental paradigm for in vivo 2Photon calcium imaging during passive sensory exposure and active behavioral task.

a) Setup for passive sensory stimulation. Head-fixed mice underwent 2Photon calcium imaging of L2/3 pyramidal neurons at both somatic and dendritic levels. During these sessions, mice were presented with unisensory stimuli (visual: drifting gratings on screen; tactile: air puff to whiskers; auditory: tone) and their multisensory combinations. Mice were not water-restricted and were not required to perform any behavioral action. This phase established baseline neuronal activity patterns before learning (passive naive).

b) Schematic representing the active behavioral task context. Following the naive phase, water restricted mice were trained on an active associative learning task (described previously) typically involving associating specific sensory cues (e.g., visual, tactile) with a reward (e.g., water delivered via a lick spout). 2Photon imaging targeted responding L2/3 neurons in relevant cortical areas, V1 and RL, during task performance and subsequent passive stimulus presentations ('passive trained' condition).

Comparing neuronal responses between 'passive naive' and 'passive trained' allowed for assessment of learning-induced changes in sensory processing at dendritic and somatic levels. Imaging was performed concurrently with stimulus presentation in both passive and active conditions. Created in BioRender.

1.1.1. Learning Globally Enhances Task-Relevant Sensory Representations within Pooled Responsive Populations

To assess how associative learning globally modulates sensory processing across neuronal populations, without selection for specific unimodal or multimodal preferences, we analyzed the average responses of all ROIs that were significantly responsive to at least one of the tested sensory modalities (visual, tactile, visuotactile, or auditory) within a given brain area, ROI type (dendrite or soma), and experimental condition (Passive Naive, MS active task, or Passive Trained) (**Figure 17**). This inclusive approach allows for tracking overall changes in sensory gain and responsiveness across learning, with auditory stimuli serving as a crucial negative control, as they were presented only during passive sessions but were not part of the active multisensory (MS) training.

The total number of ROIs contributing to these pooled population averages generally decreased from the Passive Naive to the Passive Trained state across both V1 and RL, for both dendrites and somata (e.g., V1 dendrites: $n=249$ naive vs. $n=30$ trained; RL dendrites: $n=278$ naive vs. $n=18$ trained). This indicates that the observed changes in response amplitude occur within a more refined, albeit smaller, ensemble of active units after learning.

Responses in V1:

In V1 dendrites (**Figure 17a**, top row), the Passive Naive state ($n=249$) was characterized by dominant responses to visual (mean $dF/F \pm SEM = 0.1939 \pm 0.0112$, integral = 0.5952) and visuotactile (0.1873 ± 0.0135 , integral = 0.5745) stimuli. Tactile

responses were minimal (0.0078 ± 0.0043 , integral = 0.0246), and auditory responses were negligible or slightly suppressive (-0.0135 ± 0.0045 , integral = -0.0408). During active MS task engagement ($n=7$), responses were generally low, likely reflecting the small number of dendrites broadly responsive across all presented stimuli in this sparse sample. Post-training (Passive Trained, $n= 30$), visual (0.1368 ± 0.0355 , integral = 0.4169) and visuotactile (0.1879 ± 0.0388 , integral = 0.5772) responses remained the strongest. Notably, the average tactile response showed a relative increase compared to the naive state (0.0934 ± 0.0224 , integral = 0.2888), while auditory responses remained minimal (-0.0058 ± 0.0117 , integral = -0.0166).

Whereas, somatic responses in V1 (**Figure 17b**, top row) largely mirrored this pattern. In the Passive Naive state ($n= 165$), visual (0.1034 ± 0.0112 , integral = 0.3199) and visuotactile (0.1144 ± 0.0118 , integral = 0.3491) stimuli elicited the largest somatic responses, with very weak tactile (0.0024 ± 0.0032 , integral = 0.0078) and auditory (-0.0066 ± 0.0043 , integral = -0.0201) responses. During the MS task ($n= 21$), visuotactile stimuli evoked the clearest, albeit modest, activity (0.105 ± 0.0138 , integral = 0.320). Additionally, in the Passive Trained state ($n= 94$), responses to visual (0.0508 ± 0.0097 , integral = 0.1550) and visuotactile (0.0817 ± 0.0115 , integral = 0.2489) stimuli were maintained, and tactile responses (0.0287 ± 0.0048 , integral = 0.0884) showed a slight increase over naive levels. Auditory responses remained negligible (0.0044 ± 0.0036 , integral = 0.0138).

Taken all together, in V1 we observe modest learning-induced changes in pooled responses.

Responses in RL:

A more dramatic learning induced transformation was observed in RL. In RL dendrites (**Figure 17a**, bottom row), the Passive Naive condition ($n= 276$) showed initial responsiveness primarily to visual (0.0858 ± 0.0053 , integral = 0.2639) and visuotactile (0.1057 ± 0.0057 , integral = 0.3246) stimuli, with smaller tactile (0.0229 ± 0.0032 , integral = 0.0709) and auditory (0.0043 ± 0.0025 , integral = 0.0129) responses. While during the active MS task ($n= 14$), overall activity was low, though visuotactile

responses (0.0286 ± 0.0116 , integral = 0.0891) were most apparent. Following training (Passive Trained, $n= 18$), a striking amplification occurred for task-relevant modalities: visuotactile responses became exceptionally strong (0.6901 ± 0.1495 , integral = 2.1312), and responses to visual (0.4703 ± 0.0960 , integral = 1.4497) and tactile (0.1195 ± 0.0515 , integral = 0.3665) stimuli also increased substantially compared to the naive state. Crucially, auditory responses (0.0081 ± 0.0275 , integral = 0.0195) remained low and did not exhibit potentiation, underscoring their role as a negative control.

RL somata (**Figure 17b**, bottom row) displayed a similar, less amplified, learning effect. In the Passive Naive state ($n= 157$), responses were led by visuotactile (0.1054 ± 0.0143 , integral = 0.3229) and visual (0.0902 ± 0.0134 , integral = 0.2766) stimuli, with weaker tactile (0.0213 ± 0.0052 , integral = 0.0653) and minimal auditory (-0.0001 ± 0.0045 , integral = 0.0001) activity. During the MS task ($n= 28$), visuotactile stimuli evoked the strongest responses (0.0568 ± 0.0099 , integral = 0.1745). Similarly, in the Passive Trained state ($n= 38$), a clear enhancement was observed for task-relevant stimuli: visuotactile responses (0.1470 ± 0.0298 , integral = 0.4497) were significantly potentiated, as were visual (0.0907 ± 0.0268 , integral = 0.2769) and tactile (0.0409 ± 0.0156 , integral = 0.1266) responses compared to their naive levels. Auditory responses (0.0306 ± 0.0173 , integral = 0.0930) remained modest and, importantly, did not show the same degree of amplification as the task-relevant modalities.

All in all, these results show a pronounced learning-induced amplification of task-relevant responses

The analysis of pooled responsive populations reveals that associative learning leads to a significant potentiation of average responses to task-relevant sensory stimuli (visual, tactile, and visuotactile), an effect most pronounced in the association area RL for both dendrites and somata. The stability or even slight decrease in responses to the auditory control stimulus, which was not part of the active training, strongly suggests that these observed enhancements are learning specific rather than a consequence of generalized neuronal hyperexcitability. This global amplification occurs within an overall sparser network of responsive neurons (as indicated by decreasing n values from naive to

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trained), suggesting that learning not only refines which neurons respond but also strengthens the output of those remaining responsive units to critical environmental cues. These findings highlight a fundamental aspect of learning-induced plasticity: the selective amplification of behaviorally relevant sensory representations across engaged neuronal populations.

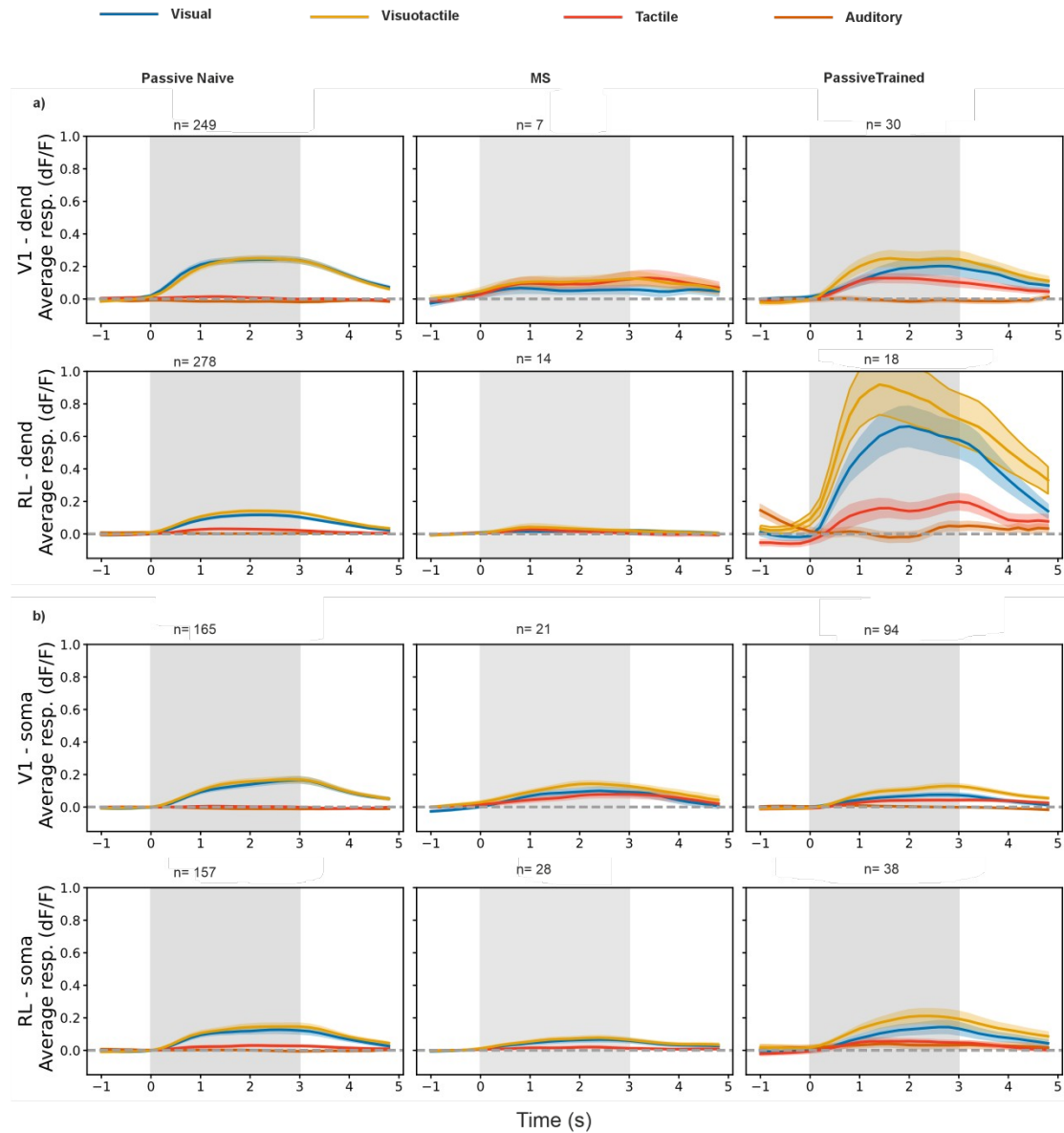


Figure 17: Associative learning enhances average population responses to task-relevant sensory stimuli, particularly in RL, while responses to control auditory stimuli remain stable.

Average dF/F responses of pooled neuronal populations in V1 and RL, comparing Passive Naive (left columns), active Multisensory (MS) task engagement (middle columns), and Passive Trained (right

columns) states. **(a)** Dendritic responses (V1-dend, top; RL-dend, bottom). **(b)** Somatic responses (V1-soma, top; RL-soma, bottom). For each plot, all ROIs that exhibited a significant response to at least one of the presented sensory modalities (visual, tactile, visuotactile, or auditory) within that specific condition and brain area were pooled to generate the average traces. Blue, yellow, pink, and dark orange traces represent average responses to visual, visuotactile, tactile, and auditory stimuli, respectively. Shaded areas around traces indicate SEM. The gray shaded region (0 to 3s) indicates the stimulus presentation period. The number of ROIs (n) contributing to each average is indicated above each plot. Note the general increase in response amplitudes to visual, tactile, and visuotactile stimuli after training (Passive Trained vs. Passive Naive), especially in RL, while auditory responses (a negative control, not present during active MS training) remain relatively stable or decrease.

1.1.1. Associative learning modulates sensory processing at the dendritic level in V1 and RL

To investigate how associative learning modulates sensory processing at the dendritic level in different cortical areas, we analyzed the average change in fluorescence (dF/F) from dendritic ROIs in V1 and RL in response to visual, tactile, and visuotactile stimuli, both before (passive naive) and after (passive trained) training. Here, we categorized dendritic ROIs based on their significant response (Mann-Whitney U test, $p < 0.01$, comparing stimulus period to baseline) to specific stimuli: visual responders, tactile responders, and visuotactile (multisensory) responders.

Visual Responders (*Figure 18a*):

ROIs identified as significantly responsive to visual stimuli were analyzed. In the naive state, these dendrites in both V1 (top-left) and RL (bottom-left) showed robust responses primarily to visual (blue trace, mean = 0.253 ± 0.019 integral = 0.778 and mean = 0.111 ± 0.008 integral = 0.342 respectively) and visuotactile (yellow trace, mean = 0.233 ± 0.018 integral = 0.715 and mean = 0.124 ± 0.008 integral = 0.382 respectively) stimuli, with minimal response to unisensory tactile stimuli (pink trace, mean = 0.009 ± 0.005 integral = 0.029 and mean = 0.018 ± 0.004 integral = 0.054

respectively). Following training, in V1 (top-right), the changes were less pronounced; responses to visual and visuotactile stimuli remained dominant but did not show a clear enhancement (mean = 0.220 ± 0.069 integral = 0.674). While the tactile response remained weak (mean = 0.066 ± 0.032 integral = 0.205). However, in RL visual responders (bottom-right) exhibited subtly enhanced responses to all three stimulus types compared to the naive state (visual mean = 0.496 ± 0.145 integral = 1.533, tactile mean = 0.083 ± 0.048 integral = 0.253, and visuotactile mean = 0.606 ± 0.227 integral = 1.876). In addition in both areas the auditory responses were very faint. Overall, training induced relatively modest changes in the response profile of visual-preferring dendrites in both areas.

Tactile Responders (*Figure 18b*):

We next examined dendrites selected for their significant response to tactile stimuli. In the naive state, these ROIs displayed generally weak and variable responses to all stimulus types in both V1 (top-left, n=11) and RL (bottom-left, n=45), consistent with the relatively small number of identified tactile responders.

V1 (visual mean = 0.083 ± 0.034 integral = 0.250, tactile mean = 0.043 ± 0.018 integral = 0.137, and tactile-visual mean = 0.067 ± 0.034 integral = 0.208) and for RL (visual mean = 0.045 ± 0.008 integral = 0.140, tactile mean = 0.060 ± 0.008 integral = 0.186, and Visuotactile mean = 0.088 ± 0.013 integral = 0.270).

However, after training, a striking difference emerged, particularly in RL. While V1 tactile responders (n= 4) showed a moderate increase in response amplitude to tactile and visuotactile stimuli (top-right, tactile mean = 0.123 ± 0.037 integral = 0.382 and visuotactile mean = 0.154 ± 0.051 integral = 0.473), RL tactile responders (n= 2) exhibited a dramatic enhancement (bottom-right). Both tactile and visuotactile stimuli evoked substantially larger responses in RL post-training compared to the naive state (tactile mean = 0.390 ± 0.252 integral = 1.218 and visuotactile mean = 0.431 ± 0.234 integral = 1.347). Responses to visual stimuli remained minimal in this population in both areas. Similarly, auditory responses were minimal (V1: auditory mean = -0.015 ± 0.013 integral = -0.044, RL: auditory mean = 0.003 ± 0.004 integral = 0.008). This

suggests that training significantly boosts the gain or recruitment of tactile responses in RL dendrites.

Visuotactile (Multisensory) Responders (*Figure 18c*):

Finally, we analyzed dendrites responsive to the combined visuotactile stimulus. In the naive state, these multisensory-responsive dendrites in both V1 (top-left, $n = 177$) and RL (bottom-left, $n = 209$) showed response profiles dominated by the visual component, resembling the visual responders (*Figure 18a*), with strong responses to visual and visuotactile stimuli and weak responses to tactile stimuli.

V1: visual mean = 0.209 ± 0.017 integral = 0.639, tactile mean = 0.006 ± 0.005 integral = 0.020, and visuotactile mean = 0.211 ± 0.016 integral = 0.646. RL: visual mean = 0.095 ± 0.006 integral = 0.292, tactile mean = 0.026 ± 0.003 integral = 0.079, and visuotactile mean = 0.118 ± 0.006 integral = 0.363.

After training, V1 dendrites in this group ($n = 25$) showed a general, moderate enhancement of responses to all three stimuli (top-right, visual mean = 0.129 ± 0.039 integral = 0.395, tactile mean = 0.079 ± 0.023 integral = 0.245, and visuotactile mean = 0.185 ± 0.044 integral = 0.569). Similar to the tactile responders, the most significant change occurred in RL (bottom-right, $n = 15$). Training led to a marked increase in the responses evoked by visual and visuotactile stimuli in these RL dendrites (visual mean = 0.508 ± 0.111 integral = 1.565, and mean = 0.821 ± 0.159 integral = 2.533 respectively). Notably, the response to the tactile component (mean = 0.152 ± 0.057 integral = 0.466) became substantially stronger post-training, indicating that learning strengthened the influence of tactile input onto these initially multisensory/visual-dominant dendrites in RL. Furthermore, in the trained state in RL, the visuotactile response often appeared larger than the response to either visual or tactile stimuli alone, suggesting enhanced multisensory integration.

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In summary, while visual responses were relatively stable or showed modest changes with training, tactile and multisensory processing underwent significant plasticity, particularly within RL. Training strongly amplified dendritic responses to tactile and visuotactile stimuli in RL, indicating a learning-induced reshaping of sensory representation and integration in RL dendrites.

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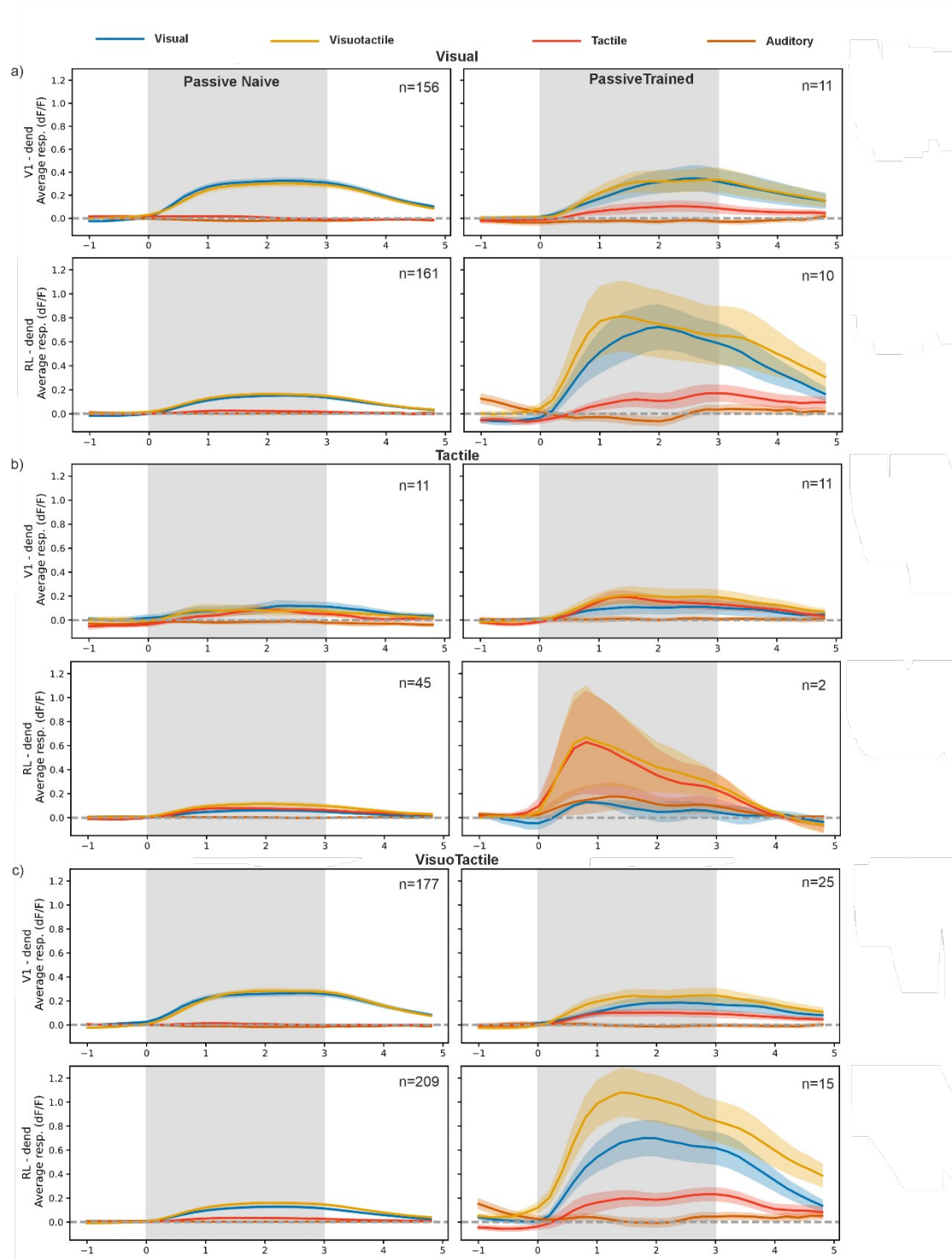


Figure 18: Training enhances tactile and multisensory dendritic responses, particularly in RL.

Average dendritic responses (dF/F) in V1; upper rows and RL; lower rows before (passive naive, left columns) and after (passive trained, right columns) associative learning.

Responses are grouped by the stimulus selectivity of the dendritic ROIs: **(a)** Visual responders **(b)** Tactile responders, and **(c)** Visuotactile (multisensory) responders. ROI selection was based on a significant increase in activity during the respective stimulus presentation compared to baseline (Mann-Whitney U test, $p < 0.01$). In addition the area under the curve was computed using the trapezoidal rule. Blue, pink, yellow and orange traces represent average somatic dF/F responses to visual, tactile, visuotactile, and auditory stimuli, respectively. Shaded areas around the trace indicate the SEM. The main grey shaded region (0 to 3 seconds) indicates the stimulus presentation period. The number of ROIs 'n' for every area and for Passive naïve and trained groups is listed on the figure.

1.1.2. Impact of learning on sensory processing in dendrites of L2/3 pyramidal neurons in V1 and RL

To further dissect the impact of learning on sensory processing, we specifically analyzed dendritic ROIs exhibiting a preferential response to one stimulus modality over the others during the stimulus presentation period (0-3s). This differs from the analysis in **Figure 18**, which included all ROIs significantly responsive to a given stimulus regardless of their responses to other stimuli. Here, ROIs were categorized as "dominant" responders if their average dF/F response during the stimulus window was greatest for either visual, tactile, or visuotactile stimuli compared to the other two conditions.

Dominant Visual Responders (**Figure 19a**):

Dendrites preferentially responding to visual stimuli were examined. As expected by the selection criteria, in the naïve state, these ROIs in both V1 (top-left, $n = 90$) and RL (bottom-left, $n = 59$) responded most strongly to visual and visuotactile likely driven by the visual component, with negligible responses to purely tactile input.

In V1: visual mean = 0.297 ± 0.026 integral = 0.915, tactile mean = 0.008 ± 0.008 integral = 0.026, and visuotactile mean = 0.232 ± 0.023 integral = 0.711. And in RL, visual mean = 0.142 ± 0.013 integral = 0.436, tactile mean = 0.013 ± 0.006 integral = 0.039, and visuotactile mean = 0.105 ± 0.012 integral = 0.321

Following training (right column), these dendrites maintained their preference for visual stimuli. In V1 (n= 2), responses to visual and visuotactile stimuli appeared somewhat enhanced compared to the naive state, and a small tactile response emerged (visual mean = 0.521 ± 0.092 integral = 1.597, tactile mean = 0.088 ± 0.064 integral = 0.273, and visuotactile mean = 0.480 ± 0.108 integral = 1.471). In RL, the post-training responses to visual stimuli were also enhanced relative to the naive state, while the tactile response remained minimal (visual mean = 0.201 ± 0.068 integral = 0.620, tactile mean = -0.020 ± 0.033 integral = -0.064, and visuotactile mean = 0.050 ± 0.028 integral = 0.157). This suggests that training amplifies existing visual responses in these preferentially tuned dendrites but does not fundamentally alter their modality preference

Dominant Tactile Responders (*Figure 19b*):

Next, we analyzed dendrites showing a preference for tactile stimuli (V1: n= 3, RL: n= 14). The number of dominant tactile responders identified in both V1 and RL using this strict criterion was very small, leading to potentially noisy average traces. In the naive passive task, V1 traces showed weak and variable responses (top-left), while RL dendrites showed very low baseline activity and weak responses overall, with a marginal preference for tactile stimuli (bottom-left). In V1: visual mean = 0.011 ± 0.031 integral = 0.032, tactile mean = 0.056 ± 0.036 integral = 0.182, and visuotactile mean = 0.009 ± 0.029 integral = 0.029. In RL visual mean = 0.011 ± 0.008 integral = 0.036, tactile mean = 0.066 ± 0.011 integral = 0.204, and visuotactile mean = 0.031 ± 0.011 integral = 0.096.

Responses remained relatively weak and variable after training, making it difficult to conclude a strong learning effect within this specific, small subpopulation identified by the exclusivity criterion with n= 0 for dominant tactile ROIs in RL (V1: visual mean = -0.032 ± 0.015 integral = -0.101, tactile mean = 0.100 ± 0.037 integral = 0.315, and visuotactile mean = 0.011 ± 0.031 integral = 0.033).

Dominant Visuotactile Responders (*Figure 19c*):

Finally, we focused on dendrites preferentially responding to the combined visuotactile stimulus. In the naive state (left column), these dendrites in both V1 ($n = 92$) and RL ($n = 140$) responded most strongly to the visuotactile stimulus, with moderate responses to visual stimuli and weaker responses to tactile stimuli. For V1: visual mean = 0.130 ± 0.019 integral = 0.399 tactile mean = 0.001 ± 0.005 integral = 0.005, and visuotactile mean = 0.184 ± 0.020 integral = 0.565. And for RL: visual mean = 0.082 ± 0.006 integral = 0.252, tactile mean = 0.025 ± 0.004 integral = 0.076, and visuotactile mean = 0.127 ± 0.008 integral = 0.390.

Post-training (right column), the preference for the visuotactile stimulus was maintained and amplified in both areas. In V1, responses to all three stimuli increased moderately ($n = 18$, visual mean = 0.100 ± 0.039 integral = 0.305, tactile mean = 0.076 ± 0.026 integral = 0.235, and visuotactile mean = 0.185 ± 0.049 integral = 0.568). However, the effect was again more pronounced in RL. Following training, RL dendrites showed markedly larger responses to the preferred visuotactile stimulus ($n = 12$, mean = 1.005 ± 0.159 integral = 3.100). Notably, the responses to both visual and tactile unisensory stimuli also increased substantially in this population compared to the naive state, suggesting that training enhances both the unisensory components and their combined multisensory representation in these RL dendrites (visual mean = 0.606 ± 0.122 integral = 1.867, and tactile mean = 0.186 ± 0.066 integral = 0.570). The post-training visuotactile response in RL often exceeded the responses to either unisensory stimulus alone, potentially indicating enhanced multisensory integration.

In summary, isolating dendritic populations based on stimulus preference reveals that associative learning primarily acts to amplify responses within existing tuning profiles, with the most significant plasticity occurring in RL. While visually-preferring dendrites showed enhancement (**Figure 19a**), the most striking plasticity was observed in RL dendrites preferring visuotactile stimuli (**Figure 19c**), where training significantly potentiated responses to both unisensory components and their combination. In contrast, the very small population of RL dendrites identified as exclusively tactile-preferring under this strict criterion did not show clear evidence of such potentiation, suggesting that strong learning-induced tactile enhancement in RL

might be more prominent in dendrites with broader tuning (*Figure 19b*) or those integrating multiple senses (*Figure 19c*).

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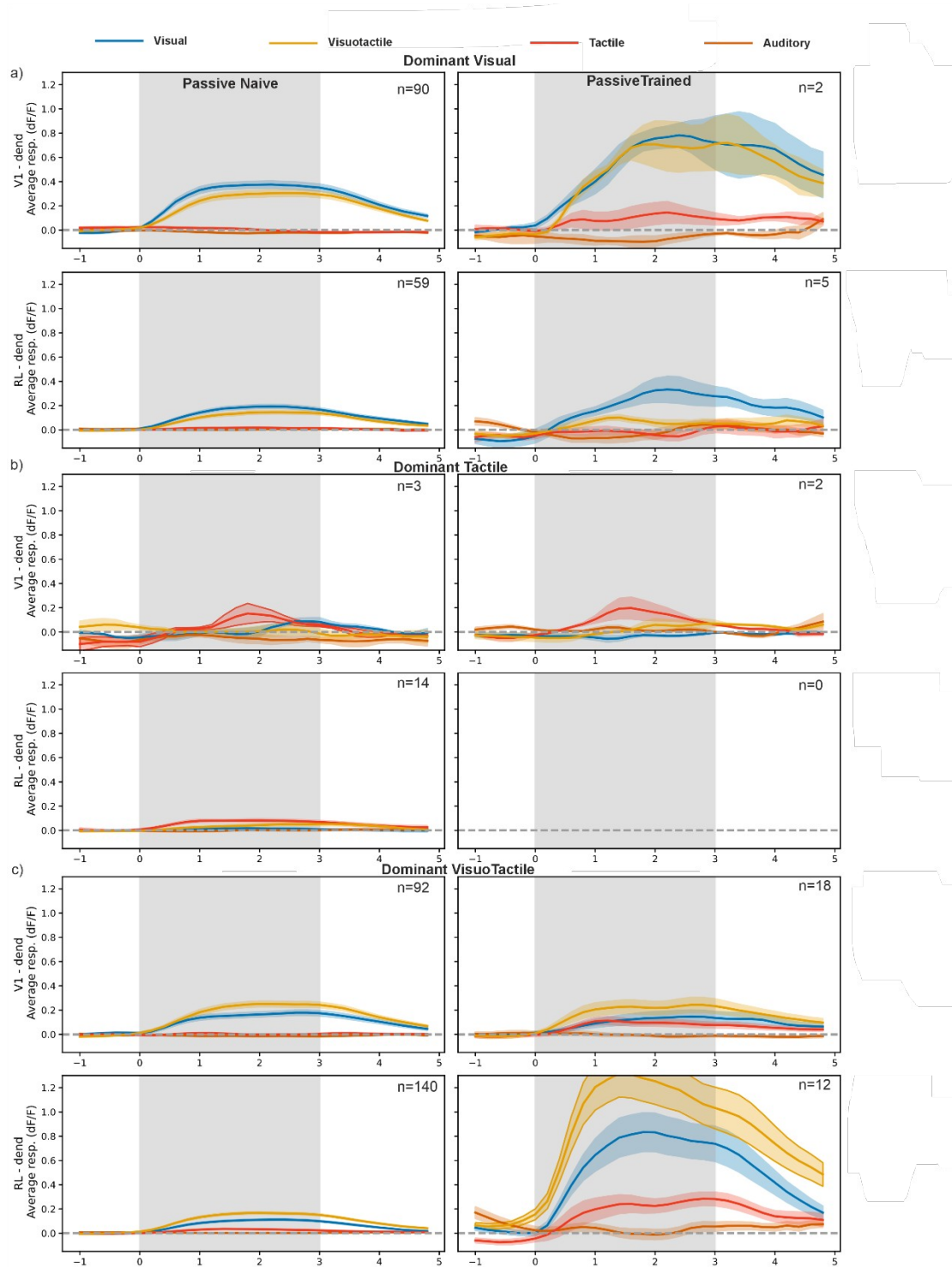


Figure 19: Training enhances responses primarily in visually and multisensory-preferring dendritic populations in RL.

Average dendritic responses (dF/F) in V1 (upper rows) and RL (lower rows) before (passive naive, left columns) and after (passive trained, right columns) associative learning, focusing on ROIs with

dominant or preferential responses. ROIs were selected based on exhibiting a stronger mean response during the stimulus period to one stimulus type compared to the other two (e.g., dominant visual: mean visual $dF/F > \text{mean tactile } dF/F$ AND mean visual $dF/F > \text{mean visuotactile } dF/F$), based on trial-averaged dF/F and Mann-Whitney U tests ($p < 0.01$). In addition the area under the curve was computed using the trapezoidal rule.

Panels show responses grouped by preference: **(a)** Dominant visual responders in V1 and RL **(b)** Dominant tactile responders in V1 and RL, and **(c)** Dominant visuotactile in V1 and RL. Blue, pink, yellow and orange traces represent average somatic dF/F responses to visual, tactile, visuotactile, and auditory stimuli, respectively. Shaded areas around the traces indicate SEM. Main grey shaded region (0-3s) marks stimulus presentation. The number of corresponding ROIs is shown 'n'.

The training induced enhancement of responses in RL dendrites preferring visual or visuotactile stimuli, while the small population of exclusively tactile-preferring RL dendrites showed fewer clear changes.

1.1.3. Associative learning modulates sensory processing at the somatic level in V1 and RL

Complementing the analysis of dendritic activity, we investigated how associative learning modulates sensory processing at the level of the neuronal soma in V1 and RL. We analyzed the average somatic calcium responses (dF/F) evoked by visual, tactile, and visuotactile stimuli before (passive naive) and after (passive trained) learning. Somatic ROIs were categorized based on their significant stimulus-evoked response (Mann-Whitney U test, $p < 0.01$, stimulus vs. baseline) to visual, tactile, or visuotactile stimuli, similar to the dendritic analysis (**Figure 18**).

Visual Responders (**Figure 20a**):

We first examined neurons identified as significantly responsive to visual stimuli. In the naive state, somatic responses in both V1 (top-left, $n = 102$) and RL (bottom-left, $n = 92$) were primarily driven by visual and visuotactile stimuli, with minimal responses to tactile stimuli. In V1: visual mean = 0.144 ± 0.016 integral = 0.437, tactile mean = -0.002 ± 0.004 integral = -0.007, and visuotactile mean = 0.148 ± 0.017 integral = 0.451.

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And in RL visual mean = 0.132 ± 0.021 integral = 0.403, tactile mean = 0.005 ± 0.006 integral = 0.015, and visuotactile mean = 0.133 ± 0.022 integral = 0.405

Following training, V1 somata showed similar responses as before training (top-right, n= 34, visual mean = 0.099 ± 0.021 integral = 0.301, tactile mean = 0.016 ± 0.006 integral = 0.048, and visuotactile mean = 0.113 ± 0.026 integral = 0.343). In contrast, RL somata exhibited a clearer enhancement of responses after training (bottom-right, n= 12), particularly for visual and visuotactile stimuli, although the response to tactile stimuli remained low (visual mean = 0.144 ± 0.027 integral = 0.439, tactile mean = 0.031 ± 0.020 integral = 0.095, and visuotactile = 0.230 ± 0.045 integral = 0.703 dF/F·s). This suggests learning potentiates visually driven somatic responses more prominently in RL than in V1 within this visual-responsive population.

Tactile Responders (*Figure 20b*):

Next, we analyzed neurons significantly responsive to tactile stimuli. In the naive state, V1 somata showed weak and variable responses to all stimulus types, though perhaps slightly favouring tactile and visuotactile inputs (top-left, n= 10, visual mean = 0.032 ± 0.025 integral = 0.098, tactile mean = 0.016 ± 0.014 integral = 0.052, and visuotactile mean = 0.060 ± 0.033 integral = 0.186). Naive RL somata showed clearer responses preferentially to tactile followed by visuotactile stimuli compared to visual stimuli (bottom-left, n= 31, visual

mean = 0.014 ± 0.013 integral = 0.044, tactile mean = 0.080 ± 0.011 integral = 0.245, and visuotactile mean = 0.050 ± 0.017 integral = 0.153). After training, V1 somatic responses remained relatively weak and showed no clear enhancement except for tactile stimuli (top-right, n= 34, visual mean = 0.023 ± 0.010 integral = 0.071, tactile mean = 0.049 ± 0.009 integral = 0.151, and visuotactile mean = 0.064 ± 0.014 integral = 0.194). However, in RL, training induced a marked potentiation of somatic responses to tactile stimuli (bottom-right, n= 5, tactile mean = 0.098 ± 0.051 integral = 0.300, and visuotactile mean = 0.045 ± 0.055 integral = 0.138). The visual response in these RL neurons remained minimal (visual mean = -0.045 ± 0.062 integral = -0.137). This indicates that associative learning significantly amplifies somatic responses to tactile inputs in V1 and RL tactile-responsive neurons.

Visuotactile (Multisensory) Responders (*Figure 20c*):

Finally, we analyzed neurons responsive to the combined visuotactile stimulus. In the naive state, these neurons in both V1 (top-left, $n = 118$) and RL (bottom-left, $n = 111$) responded most strongly to visual and visuotactile stimuli, with weaker responses to tactile stimuli, resembling the profile of visual responders (*Figure 18a*). For V1 somas: visual mean = 0.123 ± 0.014 integral = 0.372, tactile mean = 0.002 ± 0.004 integral = 0.006, and visuotactile mean = 0.136 ± 0.015 integral = 0.416 For RL somas: visual mean = 0.117 ± 0.018 integral = 0.359, tactile mean = 0.023 ± 0.006 integral = 0.072, and visuotactile mean = 0.140 ± 0.019 integral = 0.428.

After training, V1 somata in this group showed no significant changes (top-right, $n = 72$, visual mean = 0.066 ± 0.012 integral = 0.201, tactile mean = 0.030 ± 0.005 integral = 0.091, and visuotactile mean = 0.102 ± 0.014 integral = 0.309). In contrast, RL somata exhibited a significant enhancement of responses following training (bottom-right, $n = 22$). Responses to visuotactile stimuli were strongly potentiated. Visual responses were also enhanced, in addition to the response to tactile stimuli (visual mean = 0.127 ± 0.026 integral = 0.389, tactile mean = 0.029 ± 0.014 integral = 0.089, and visuotactile mean = 0.176 ± 0.033 integral = 0.539). This suggests that training strengthens the representation of both the constituent unisensory inputs and the combined multisensory stimulus at the somatic level in RL neurons initially identified as multisensory-responsive.

In summary, associative learning induces plasticity in somatic sensory responses, mirroring patterns observed dendritically but with generally lower dF/F amplitudes. While visual responses showed some enhancement, particularly in RL, the most pronounced learning effect at the somatic level was the significant potentiation of responses to tactile and visuotactile stimuli within RL neurons responsive to these modalities. This highlights a key role for RL in integrating and amplifying behaviorally relevant tactile information after associative learning.

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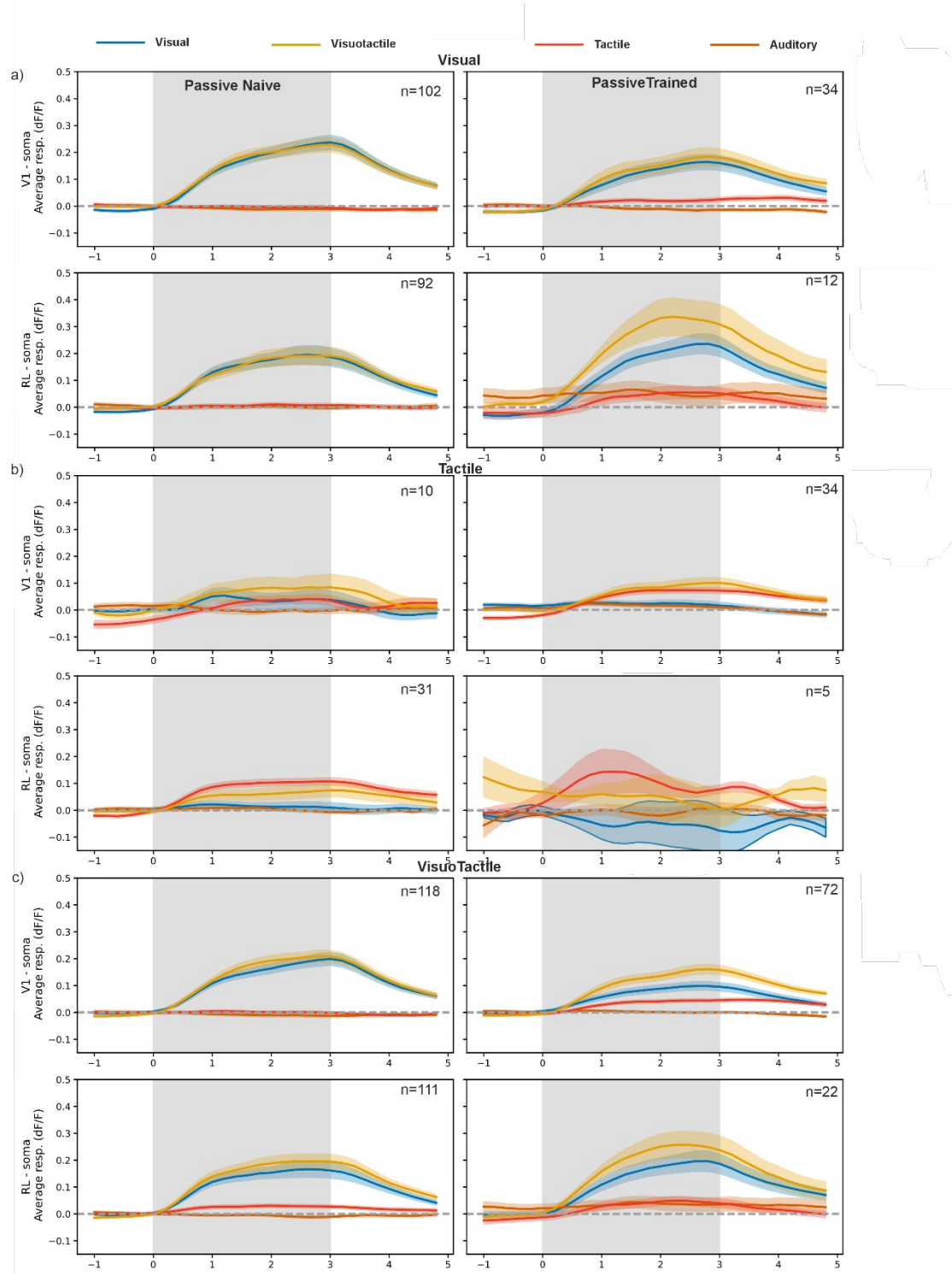


Figure 20: Training enhances somatic sensory responses, particularly for multisensory stimuli in RL.

Average somatic responses (dF/F) in V1; upper rows and in RL; lower rows before (passive naive, left columns) and after (passive trained, right columns) associative learning.

Responses are grouped by the stimulus selectivity of the somatic ROIs: **(a)** Visual responders in V1 and RL **(b)** Tactile V1 and RL, and **(c)** Visuotactile (multisensory) responders V1 and RL. ROI selection was based on a significant increase in somatic activity during the respective stimulus presentation compared to baseline (Mann-Whitney U test, $p < 0.01$). In addition the area under the curve was computed using the trapezoidal rule. Blue, pink, yellow and orange traces represent average somatic dF/F responses to visual, tactile, visuotactile, and auditory stimuli, respectively. Shaded areas around the trace indicate the SEM. The main grey shaded region (0 to 3 seconds) indicates the stimulus presentation period. 'n' represents the number of ROIs in each area and condition.

The training induced enhancement of somatic responses, especially evident for visuotactile stimuli within the RL area.

1.1.1. Impact of learning on sensory processing in somatas of L2/3 pyramidal neurons in V1 and RL

To isolate learning-induced changes within distinct functional subpopulations at the somatic level, we analyzed neurons exhibiting a preferential response to one specific stimulus modality over the others during the stimulus presentation window (0-3s). This "dominant" selection criterion contrasts with the analysis in *Figure 20*, which included all neurons significantly responsive to a stimulus.

Dominant Visual Responders (*Figure 21a*):

Neurons preferentially responding to visual stimuli at the soma were examined. In the naive state, these neurons in both V1 (top-left, $n = 49$) and RL (bottom-left, $n = 48$) responded strongly to visual and visuotactile stimuli, consistent with their selection, while showing minimal tactile responses. In V1: visual mean = 0.164 ± 0.024 integral = 0.500, tactile mean = -0.007 ± 0.005 integral = -0.023, and visuotactile mean = 0.118 ± 0.022 integral = 0.361. Whereas in RL, visual mean = 0.149 ± 0.024 integral = 0.456, tactile mean = 0.003 ± 0.004 integral = 0.010, and visuotactile mean = 0.098 ± 0.018 integral = 0.299.

Following training (right column), these somatic responses were stable with no significant changes in both areas. V1 neurons showed similar visual and visuotactile

responses, and a small tactile response emerged ($n= 13$, visual mean = 0.138 ± 0.029 integral = 0.420, tactile mean = 0.018 ± 0.011 integral = 0.053, and visuotactile mean = 0.094 ± 0.027 integral = 0.287). RL neurons ($n= 3$) exhibited significantly enhanced visual responses post-training, while tactile and visuotactile responses remained the same (visual mean = 0.178 ± 0.046 integral = 0.545, tactile mean = -0.010 ± 0.006 integral = -0.028, and visuotactile mean = 0.107 ± 0.039 integral = 0.331). This indicates that learning amplifies visually driven somatic output in neurons already tuned to visual stimuli in both V1 and RL.

Dominant Tactile Responders (*Figure 21b*):

We next analyzed the relatively small populations of neurons showing a somatic preference for tactile stimuli (V1: $n= 3$, RL: $n= 21$). In the naive state, V1 neurons (top-left) displayed weak and variable responses to all stimulus types (visual mean = -0.012 ± 0.039 integral = -0.040, tactile mean = 0.015 ± 0.023 integral = 0.051, and visuotactile mean = -0.030 ± 0.040 integral = -0.092). RL neurons (bottom-left) showed a clearer but weak, preference for tactile stimuli (visual mean = -0.013 ± 0.012 integral = -0.039, tactile mean = 0.085 ± 0.015 integral = 0.261, and visuotactile mean = 0.017 ± 0.016 integral = 0.053). After training, V1 tactile-preferring neurons showed relatively little change, with responses becoming more robust for tactile stimuli (top-right, $n= 14$, visual mean = -0.008 ± 0.012 integral = -0.025, tactile mean = 0.057 ± 0.017 integral = 0.175, and visuotactile mean = 0.020 ± 0.021 integral = 0.061). However, in RL, training induced a clear and substantial potentiation of the somatic response specifically to tactile stimuli in these tactile-preferring neurons (bottom-right, $n= 2$, visual mean = -0.163 ± 0.105 integral = -0.496, tactile mean = 0.194 ± 0.087 integral = 0.596, and visuotactile mean = -0.068 ± 0.058 integral = -0.215). This result contrasts with the lack of clear enhancement in the very small exclusively tactile-preferring dendritic population (*Figure 19*), suggesting that learning-induced amplification of tactile signals in RL is robustly reflected in the somatic output of tactile-specialized neurons.

Dominant Visuotactile Responders (*Figure 21c*):

Finally, we focused on neurons preferentially responding to the combined visuotactile stimulus at the soma. In the naive state (left column), these neurons in both V1 and RL responded most strongly to the visuotactile stimulus, with weaker but present responses to visual stimuli. In V1: $n = 67$, visual mean = 0.103 ± 0.018 integral = 0.311, tactile mean = 0.005 ± 0.005 integral = 0.016, and visuotactile mean = 0.155 ± 0.020 integral = 0.473. Whereas in RL, $n = 56$, visual mean = 0.099 ± 0.027 integral = 0.305, tactile mean = 0.018 ± 0.009 integral = 0.056, and visuotactile mean = 0.172 ± 0.034 integral = 0.527.

Post-training (right column), the preference for the visuotactile stimulus was maintained and amplified, particularly in RL. V1 neurons showed a constant trend in responses to all stimuli ($n = 52$, visual mean = 0.053 ± 0.013 integral = 0.163, tactile mean = 0.028 ± 0.005 integral = 0.087, and visuotactile mean = 0.109 ± 0.017 integral = 0.332). In RL, training led to markedly larger somatic responses to the preferred visuotactile stimulus ($n = 13$, visuotactile mean = 0.217 ± 0.044 integral = 0.663). Responses to the unisensory visual and tactile components were also clearly enhanced compared to the naive state (visual mean = 0.098 ± 0.027 integral = 0.299, tactile mean = 0.033 ± 0.019 integral = 0.104), indicating that learning strengthens the somatic representation of both the individual sensory inputs and their combined multisensory integration in these RL neurons.

In summary, analyzing exclusively responding somatic populations confirms that associative learning enhances sensory representations according to their initial tuning, with the most pronounced effects occurring in RL. Learning amplified visually-driven somatic output in visual neurons. Crucially, it strongly potentiated somatic responses to tactile stimuli in RL dominant tactile neurons, and enhanced both unisensory and multisensory responses in RL neurons specialized for visuotactile integration. These somatic findings largely mirror dendritic changes but notably reveal a clear enhancement in the dominant tactile RL population, reinforcing the role of RL somatic

output in processing behaviorally relevant tactile and multisensory information after learning.

Results

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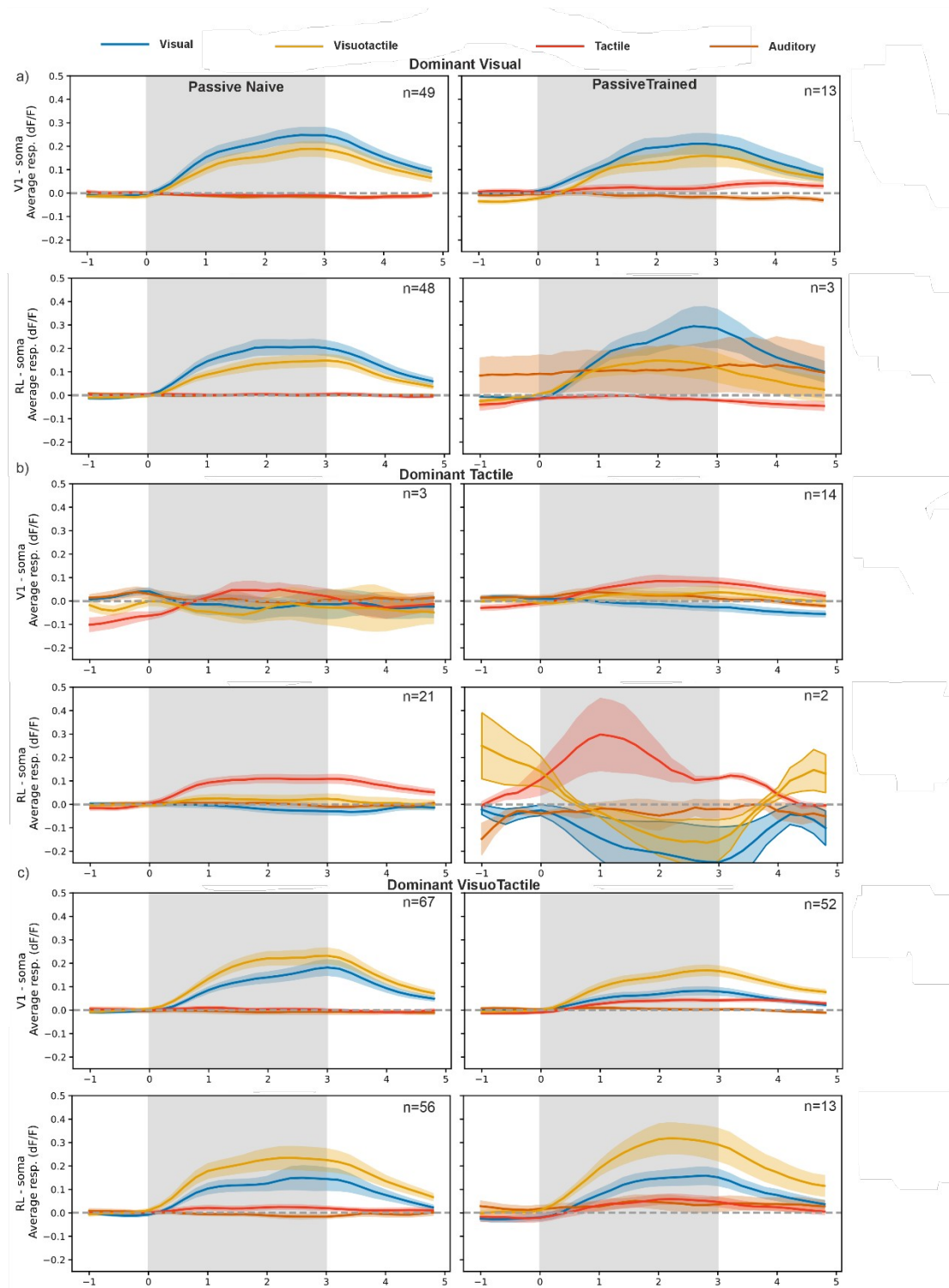


Figure 21: Training enhances somatic responses in modality-preferring neuronal populations, particularly in RL.

Average somatic responses (dF/F) in V1 (upper rows) and RL (lower rows) before (passive naive, left columns) and after (passive trained, right columns) associative learning, focusing on neurons with dominant or preferential somatic responses. Somatic ROIs were selected based on exhibiting a stronger mean response during the stimulus period (0-3s) to one stimulus type compared to the other two (e.g., dominantvisual: mean visual $dF/F >$ mean tactile dF/F AND mean visual $dF/F >$ mean visuotactile dF/F). The number of dominant ROIs is presented as 'n'.

Panels show responses grouped by preference: (a) Dominant visual responders in V1 and RL, (b) Dominant tactile responders in V1 and RL, and (c) Dominant visuotactile responders in V1 and RL. Blue, pink, yellow and orange traces represent average somatic dF/F responses to visual, tactile, visuotactile, and auditory stimuli, respectively. Shaded areas around the trace indicate SEM. In addition the area under the curve was computed using the trapezoidal rule. The grey shaded region (0-3s) marks stimulus presentation whereas.

Training notably boosts somatic responses in RL neurons preferring tactile or visuotactile stimuli.

1.1.1. Learning Induces a Sparsification and Reorganization of Responsive Neuronal Populations in V1 and RL

To understand how associative learning alters the landscape of sensory-responsive neurons and their subcellular compartments, we quantified the number and overlap of ROIs (dendrites and somata) in V1 and RL that exhibited significant calcium responses to visual, tactile, or tactile-visual stimuli before (Passive Naive) and after (Passive Trained) behavioral training (Figure 22). This analysis aimed to determine whether learning-induced changes in sensory processing are primarily driven by an increase in the recruitment of responsive units, a refinement leading to fewer but potentially more specifically tuned units, or a combination thereof.

A striking overall observation across both brain areas (V1 and RL) and ROI types (dendrites and somata) was a substantial reduction in the total number of significantly responsive ROIs after associative learning (Figure 22, compare panels 'a' and 'b' within each main panel A-D). This general decrease suggests that learning does not simply

recruit more neurons to respond to the presented stimuli, but rather leads to a sparsification or refinement of sensory representations, where fewer neuronal elements exhibit suprathreshold responses during passive stimulation post-training.

RL: Dendritic and Somatic Reorganization

In RL dendrites (**Figure 22a**), the total number of responsive units decreased dramatically from 265 in the naive state to 25 after training. In the naive state, a large proportion of dendrites responded to visual stimuli either exclusively (16.2%, $n=43$) or in combination with tactile-visual (visuotactile) stimuli (35.5% visual and tactile-visual; 7.5% all three). After training, while the absolute numbers dropped, the *proportional* representation within the remaining responsive dendrites shifted. For instance, dendrites responsive *only* to tactile stimuli increased their share from 8.3% ($n=22$) to 12.0% ($n=3$), and those responsive to tactile and tactile-visual stimuli (but not visual alone) increased from 6.4% ($n=17$) to 16.0% ($n=4$). The proportion responsive to all three modalities decreased from 7.5% ($n=20$) to 4.0% ($n=1$).

A similar, drastic reduction in total responsive ROIs was observed for RL somata (**Figure 22b**), from 165 naive to 25 trained. In the naive state, a significant portion was responsive to visual stimuli (18.2% exclusive; 35.8% visual & tactile-visual; 8.5% all three). Post-training, the landscape of the remaining responsive somata changed markedly. The proportion of somata responsive to tactile-visual (visuotactile) increased substantially from 21.8% ($n=36$) to 36.0% ($n=9$), becoming the largest single category. Concurrently, the proportion of somata responsive to all three stimuli (visual, tactile, and tactile-visual) decreased from 8.5% ($n=14$) to 0% ($n=0$), suggesting that tactile-visual responses in the trained state became more integrated at the somatic level post-training.

V1: Dendritic and Somatic Reorganization

In V1 dendrites (**Figure 22c**), the total number of responsive units also decreased substantially, from 254 naive to 28 trained. Before training, V1 dendrites were predominantly responsive to visual stimuli (26.0% exclusive; 37.4% visual & tactile-

visual). After training, among the diminished population, the proportion of dendrites responsive to all three stimuli increased from a very small fraction 1.6% (n= 4) to 14.3% (n= 4). The proportion exclusively responsive to visual stimuli decreased from 26.0% (n= 66) to 10.7% (n= 3), and those to tactile-visual (visuotactile) stimuli from 29.1% (n= 74) to 21.4% (n= 6).

For V1 somata (**Figure 22d**), the reduction in total responsive units was also significant, from 203 naive to 97 trained, though less dramatic than in RL or V1 dendrites. Before training, visual (20.7% exclusive; 35.5% visual & tactile-visual) and tactile-visual (30.0% exclusive visuotactile) responsive somata were prevalent. After training, the proportion of somata exclusively responsive to tactile stimuli increased notably from 8.9% (n= 18) to 12.4% (n= 12). The proportion exclusively responsive to visual stimuli decreased (20.7% to 12.4%), while the exclusively tactile-visual (visuotactile) proportion remained relatively stable (30.0% to 28.9%). The overlap representing responsiveness to all three modalities also increased proportionally from a small base 2.5%, n=5 to 6.2% n=6.

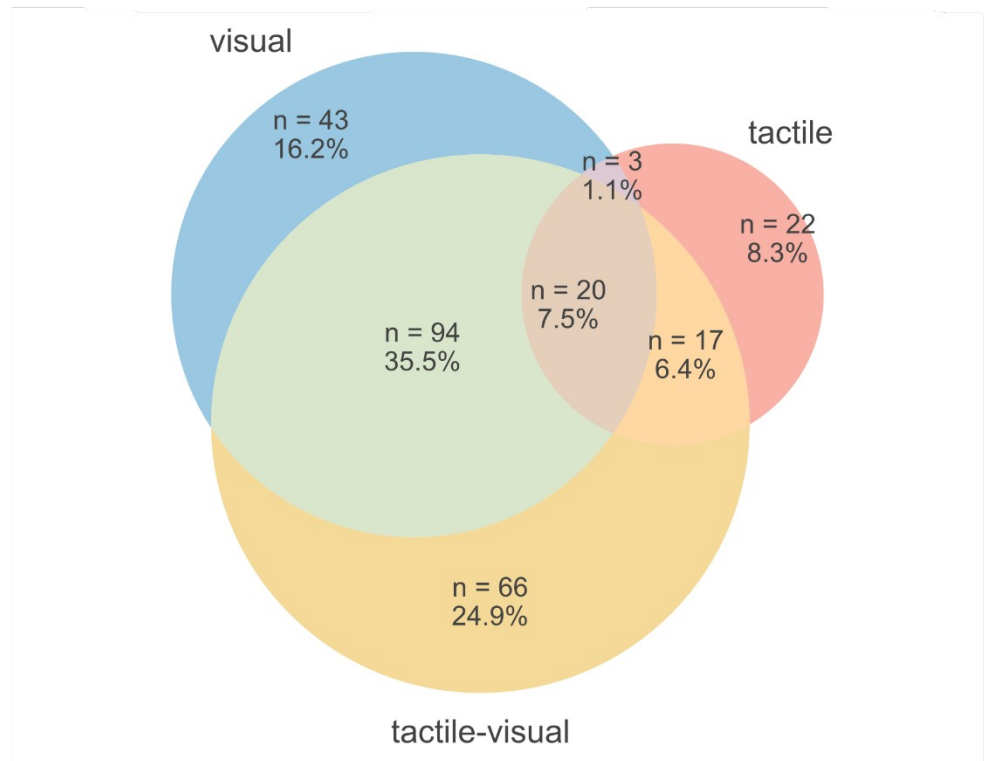
Collectively, these Venn diagrams reveal that associative learning leads to a significant sparsification of responsive neuronal elements (both dendrites and somata) in V1 and RL during passive sensory stimulation. This reduction in the sheer number of responsive units argues against a simple model where learning merely increases the quantity of neurons engaged by sensory stimuli. Instead, it suggests a refinement process. The observed shifts in the *proportions* of remaining responsive units particularly the increased proportional representation of tactile-responsive and multisensory integrating units in RL, and tactile-responsive units in V1 somata; imply that learning may sharpen tuning, prune less relevant responses, and potentially strengthen the representation of behaviorally significant cues within a smaller, more specialized ensemble of neurons. This sparsification, combined with the previously observed learning-induced enhancement of response *amplitudes* in specific populations (**Figure 18, Figure 19, Figure 20, and Figure 21**), points towards a more efficient and robust encoding strategy post-learning, where fewer neurons convey stronger and potentially more distinct information about the sensory environment.

Results

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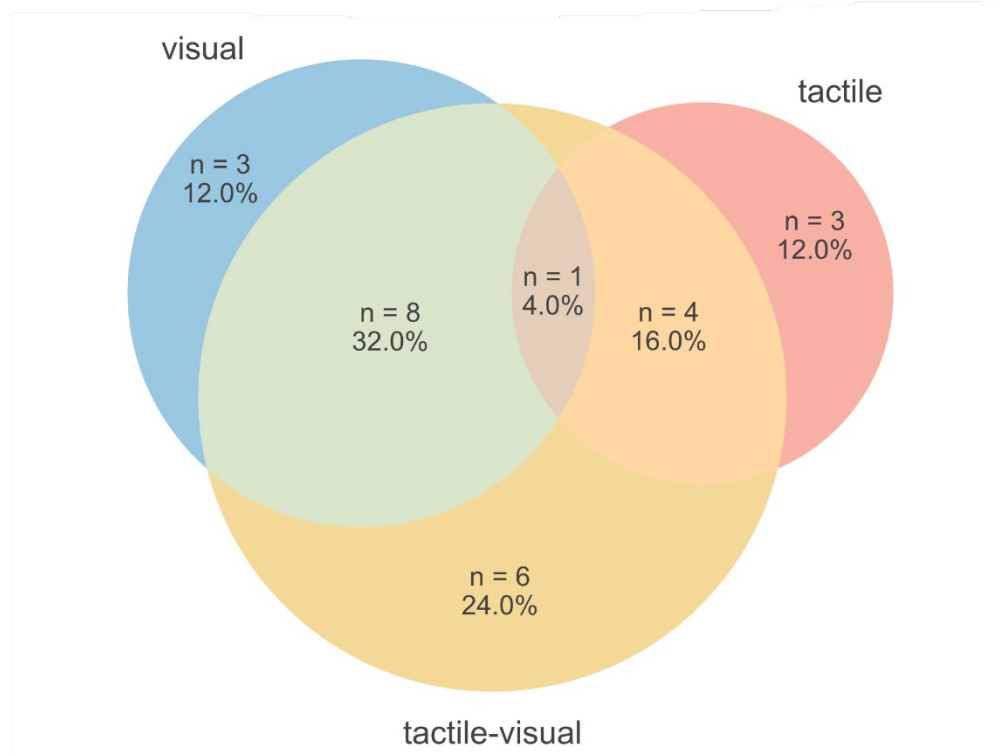
Dendrites in RL Passive Naive

a.1)



a.2)

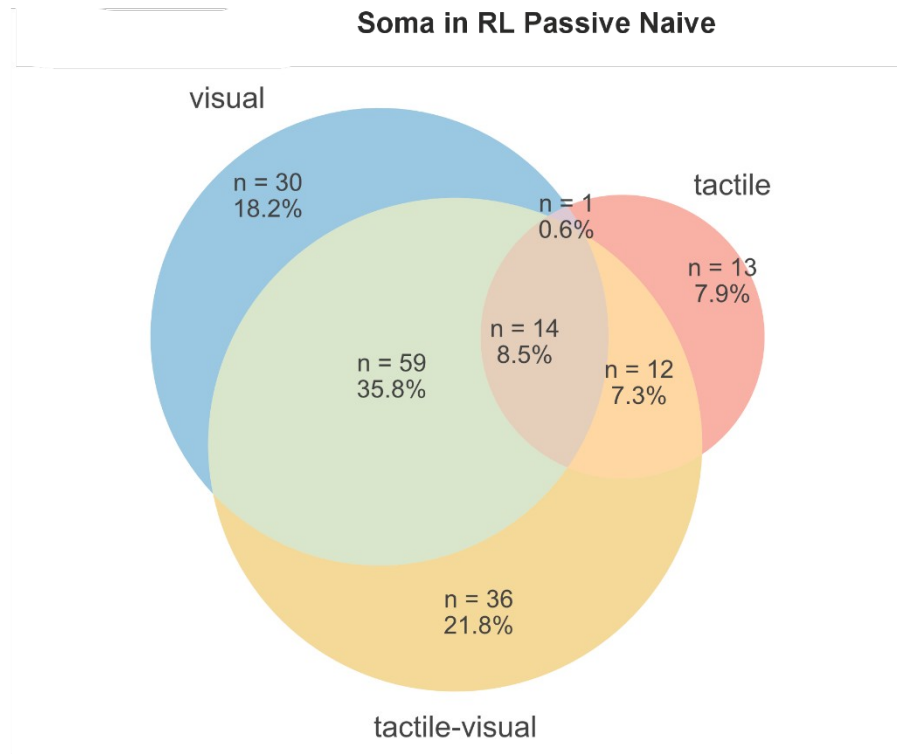
Dendrites in RL Passive Trained



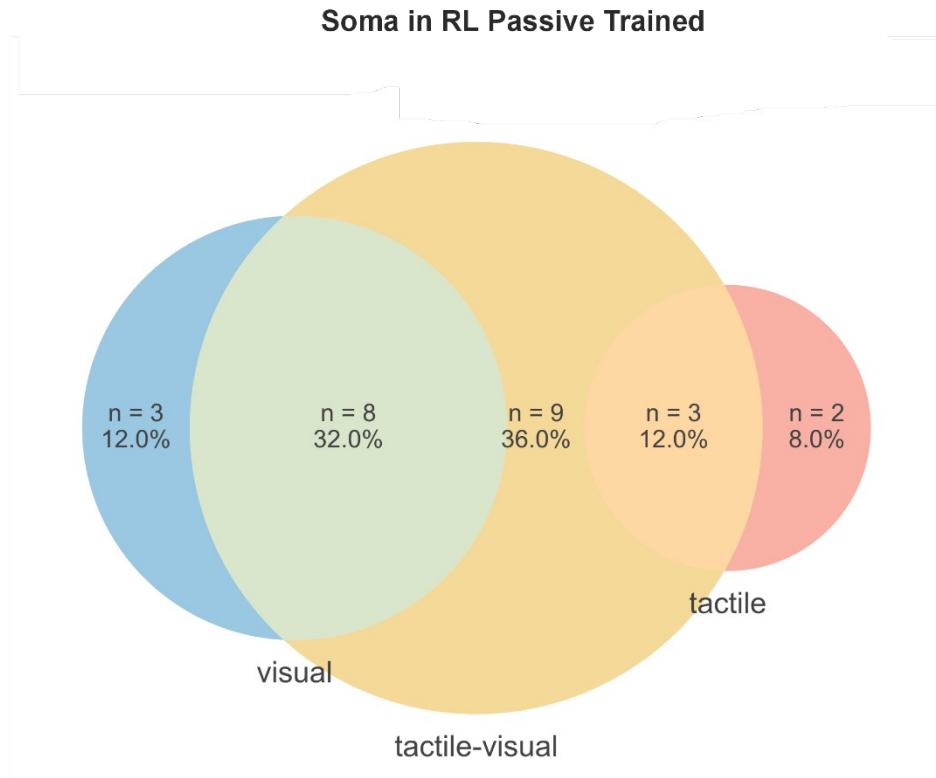
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b.1)



b.2)

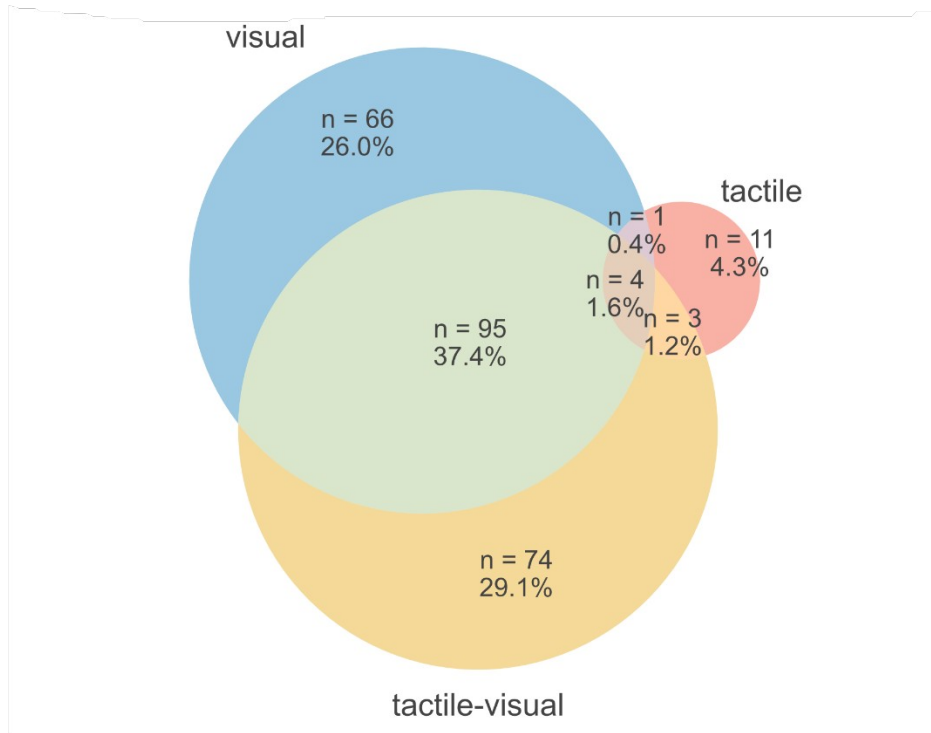


Results

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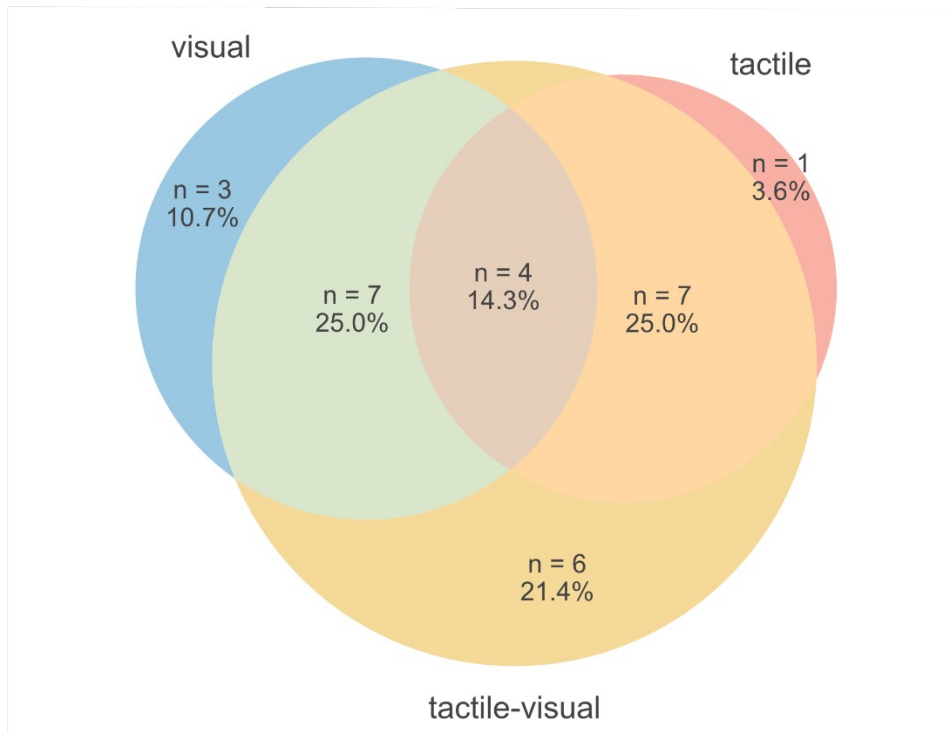
Dendrites in V1 Passive Naive

c.1)



Dendrites in V1 Passive Trained

c.2)

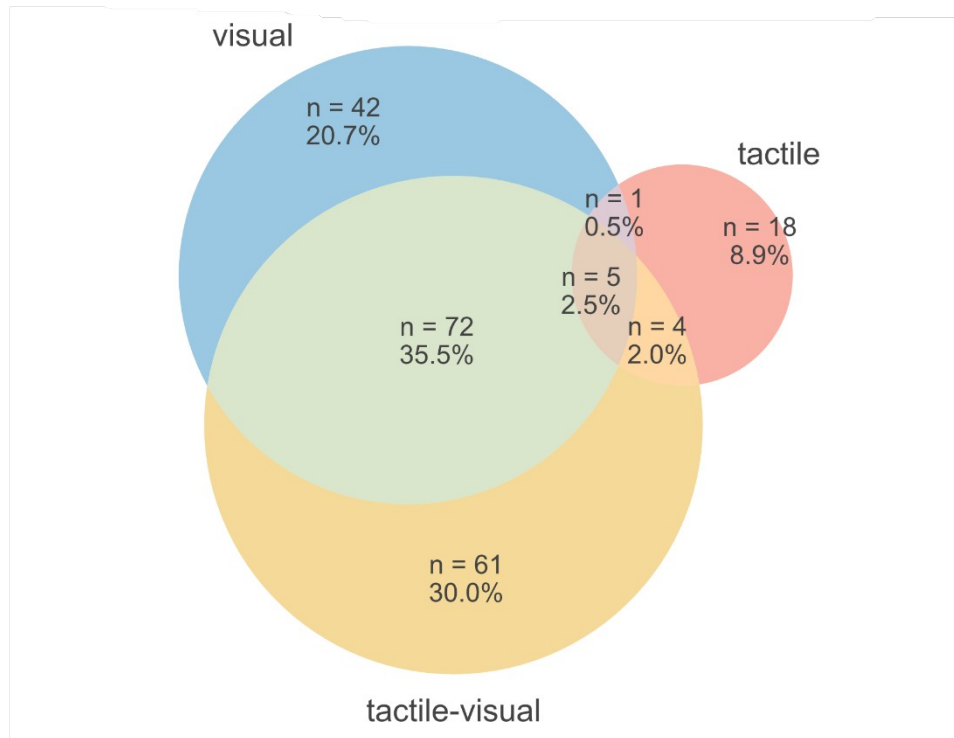


Results

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d.1)

Soma in V1 Passive Naive



d.2)

Soma in V1 Passive Trained

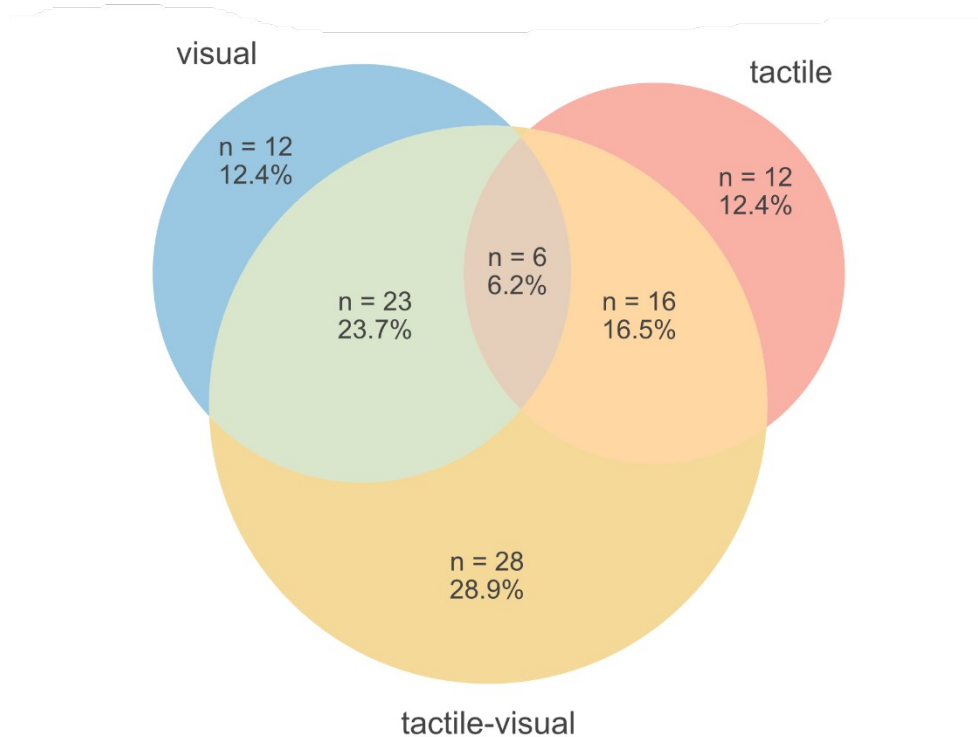


Figure 22: Associative learning leads to a reduction and redistribution of responsive dendritic and somatic populations in V1 and RL.

Venn diagrams illustrating the number (n) and percentage (%) of dendritic or somatic ROIs significantly responsive to visual (blue circle), tactile (red circle), and/or tactile-visual (yellow circle) stimuli. Comparisons are shown for **(a)** Dendrites in RL, **(b)** Soma in RL, **(c)** Dendrites in V1, and **(d)** Soma in V1. Within each main panel, subpanel (.1) depicts the distribution in the 'Passive Naive' state (before training), and subpanel (.2) depicts the 'Passive Trained' state (after associative learning). Numbers indicate the absolute count of ROIs in each segment, and percentages are relative to the total number of responsive ROIs depicted within that specific Venn diagram (i.e., sum of all segments in that diagram). Note the pronounced overall decrease in the total number of responsive ROIs after training across most conditions, alongside shifts in the proportions of unisensory-specific versus multisensory (overlapping) responsive units, particularly in RL.

2. Discussion

The perception of our environment rarely relies on a single sensory modality. Instead, the brain seamlessly integrates information from multiple senses to form a coherent and robust representation of the external world, enabling adaptive behavior (Stein & Meredith, 1993; Stein & Stanford, 2008). While the behavioral benefits of multisensory integration, such as enhanced detection, discrimination, and reaction times, are well-documented (Körding et al., 2007; Rowland, 2008), the underlying neural mechanisms, particularly at the cellular and subcellular levels within the neocortex, remain incompletely understood. This thesis aimed to investigate the anatomical basis and functional implementation of visuotactile integration in the mouse parietal association cortex, specifically the rostrolateral visual area, focusing on the role of Layer 2/3 pyramidal neurons and their dendrites during sensory processing and goal-directed behavior. Using a combination of viral tracing, chronic widefield and 2Photon calcium imaging, and behavioral paradigms, we characterized the circuitry involving RL, examined population-level neuronal correlates of MSI, and investigated learning induced plasticity in somatic and dendritic compartments

1.1. Anatomical Substrates for Visuotactile Integration in RL

A fundamental prerequisite for MSI is the convergence of inputs from different sensory modalities onto common neural structures. Our retrograde tracing experiments confirmed that RL receives inputs from a distributed network, including both primary visual and primary somatosensory cortices, as well as associated thalamic nuclei (**Figure 6**). While overall fluorescence intensity from projecting areas did not significantly differ between cortical and thalamic sources, quantification of retrogradely labeled somata revealed the highest density of projecting neurons resided in V1, followed by S1, with significantly lower densities in the visual (likely LGN/LP)

and somatosensory (likely VPM/VPL) thalamus. This suggests substantial direct cortico-cortical pathways converging onto RL, consistent with its role as a higher-order association area (Olcese et al., 2013; Wang & Burkhalter, 2007). Although, the density of directly projecting cortical neurons, particularly from V1, was higher than from the thalamus, the presence of significant thalamic input is functionally crucial. This finding indicates that sensory processing within RL, particularly visual processing, is not strictly hierarchical or limited to inputs relayed serially through V1. Instead, RL is embedded within a more complex network involving thalamo-cortical loops.

Specifically, the labeling observed in visual thalamic nuclei, likely encompassing the Lateral Posterior nucleus and potentially parts of the Lateral Geniculate Nucleus, provides a direct pathway for visual information to reach RL, potentially bypassing or complementing the V1 pathway. The LP, often considered a higher-order thalamic nucleus, receives input from both V1 and the superior colliculus and projects broadly to associative cortical areas, including parietal regions like RL (Beltramo et al., 2013; Roth et al., 2016). Such thalamic inputs can convey different types of visual information (e.g., related to saliency or visuomotor integration from the colliculus) or exert modulatory influences on cortical processing (Saalmann & Kastner, 2011; Sherman & Guillery, 2002).

Similarly, the input from somatosensory thalamic nuclei (VPM/VPL) provides a parallel route for tactile information, converging with the cortico-cortical S1 input. Therefore, the convergence of both direct cortico-cortical projections (V1 to RL, S1 to RL) and thalamo-cortical projections (Visual Thalamus to RL, Somatosensory Thalamus to RL) underscores RL's position as a multimodal integration hub receiving processed sensory information through multiple, potentially functionally distinct, anatomical streams. This complex input structure likely contributes to the sophisticated integration capabilities observed in RL during multisensory tasks.

Further investigation using dual anterograde tracing provided critical insights into the laminar organization of these converging inputs within RL (**Figure 8**). We observed a striking difference in the targeting patterns of V1 and S1 projections. S1 axons

predominantly innervated the superficial layers (L1 and L2/3) and mid-layers of RL, whereas V1 axons primarily targeted mid-to-deep layers (L4-L6). Notably, S1 projections were significantly stronger than V1 projections specifically within the superficial layers. This laminar segregation is highly significant in the context of cortical microcircuit organization and dendritic computation. L2/3 pyramidal neurons possess elaborate apical dendrites extending into L1, which are known to receive long-range associative, feedback, and modulatory inputs, while basal and proximal apical dendrites receive more feedforward information (Harris & Mrsic-Flogel, 2013; M. Larkum, 2013; Petreanu et al., 2009). The strong S1 input to superficial RL layers positions it ideally to interact with the apical tufts of L2/3 neurons, a primary site for non-linear dendritic integration via mechanisms like NMDA and Ca²⁺ spikes (M. E. Larkum et al., 1999). Conversely, V1 input targeting deeper layers might engage different dendritic compartments or interneuron populations. This anatomical arrangement, consistent with principles of cortical organization where L1 receives feedback and associative inputs (Harris & Mrsic-Flogel, 2013), provides a clear structural basis for the differential processing and potential non-linear integration of visual and tactile information within individual RL neurons, a hypothesis directly tested by our functional imaging data.

Interestingly, our anterograde tracing also revealed an asymmetry in direct connections between V1 and S1, with V1 projections to S1 being significantly stronger and more widespread across layers than S1 projections to V1 (**Figure 7**). This suggests a potentially stronger influence of visual information on primary somatosensory processing than vice versa, at least via direct pathways, consistent with findings in other species (Falchier et al., 2002; Iurilli et al., 2012).

1.2. Population-Level Correlates of MSI in RL during Behavior

Previous work in our lab using widefield imaging during a visuotactile evidence accumulation task revealed supra-linear enhancement of population activity in RL during multisensory trials, correlating with improved behavioral performance ((Nabbefeld, 2022); **Figure 3**). In this thesis, we extended these findings using 2Photon calcium imaging to examine single-cell responses in V1, S1, and RL during a similar discrimination task (**Figure 10, Figure 12**). Behavioral results confirmed MSI benefits, with mice exhibiting steeper psychometric functions during multisensory trials compared to unisensory conditions (**Figure 10d**), aligning with optimal cue integration principles (Ernst & Banks, 2002; Gu et al., 2008).

At the neural level, our initial 2P imaging data confirmed RL as a key hub for visuotactile processing. While V1 and S1 populations showed strong responses primarily to their preferred modality (with some cross-modal responses, particularly visual responses in S1), RL exhibited robust population responses to visual, tactile, and multisensory stimuli (**Figure 12b**). Functional classification revealed distinct neuronal populations within RL preferentially responding to visual, tactile, or potentially multisensory conditions (**Figure 12c**). The presence of substantial visual- and tactile-responsive populations in RL underscores the convergence observed anatomically.

However, interpreting the role of the 'MS-preferring' cells identified by our initial classification required caution. In the presented average traces (**Figure 12c**), these MS-classified cells in RL did not exhibit clear supra-linear summation or even enhancement (MS response > max unisensory response) compared to the responses of the same cell group to unisensory stimuli. This apparent discrepancy with prior widefield findings and the behavioral enhancement could arise from several factors, including averaging across heterogeneous populations or integration rules potentially occurring within subcellular compartments like dendrites (Choi et al., 2018; L. M. Palmer et al., 2014; Stein & Stanford, 2008). Firstly, widefield signals reflect the summed activity of vast, potentially heterogeneous populations, including neuropil, which might obscure or

average out nuanced single-cell integration rules (Musall et al., 2019). Secondly, our cell classification based on average responses might group neurons with diverse integration properties (supra-additive, additive, sub-additive) (Choi et al., 2018; Stein & Stanford, 2008), potentially masking enhancement effects when averaged. Supra-additivity, often observed for weak stimuli according to the principle of inverse effectiveness (Stein & Meredith, 1993), might occur only under specific stimulus conditions or trial types within the task, which averaging across all trials might obscure. Thirdly, true non-linear integration underlying behavioral benefits might occur within specific subcellular compartments, like dendrites, and may not always be linearly reflected in somatic calcium signals (L. M. Palmer et al., 2014; G. J. Stuart & Spruston, 2015), a notion central to this thesis. Finally, MSI enhancement at the population level could also emerge from network dynamics, such as changes in correlated activity or modulation of neuronal variability (Fetsch et al., 2012; Ni et al., 2018), rather than solely relying on supra-linear firing rate increases in individual neurons.

1.3. Learning effects on neuronal responses

To directly address how learning shapes responses at the cellular and subcellular levels, we compared activity patterns before (passive naive) and after (passive trained) associative learning. Our analyses of dendritic activity (**Figure 18, Figure 20**) revealed profound learning induced plasticity, particularly in RL. When considering all dendrites significantly responsive to a given modality, we observed a striking enhancement of responses to tactile and visuotactile stimuli in RL following training. While visual responders showed only modest changes, the amplification of tactile-driven responses in RL tactile and multisensory dendrites suggests that learning significantly increases the gain or influence of tactile inputs onto RL L2/3 neurons. This aligns with the anatomical finding of strong S1 input to superficial layers containing apical dendrites, suggesting these inputs are significantly potentiated through learning (Holtmaat & Svoboda, 2009; Xu et al., 2012). These findings in RL build upon previous work by (Nabbefeld, 2022), who identified RL as a critical hub for visuotactile integration,

demonstrating both behavioral improvements and neuronal response enhancement to multisensory stimuli. Our data extend these observations by pinpointing specific learning-induced potentiation of unisensory (tactile) and multisensory responses at both dendritic and somatic levels within RL, providing a cellular substrate for its role in learned multisensory associations.

Further refining this analysis by selecting dendrites with dominant preference for one modality (**Figure 19, Figure 21**) provided additional nuance. While dominant visual and dominant visuotactile populations in RL showed clear response enhancement post-training, the very small population identified as exclusively tactile-preferring did not exhibit such potentiation at the dendritic level. This could suggest that strong dendritic enhancement of tactile responses is most prominent in neurons that also integrate visual information (the larger visuotactile group) or have broader tuning, or that the strict exclusivity criterion isolates a particularly small or variable dendritic subpopulation.

Analysis of somatic responses largely mirrored the dendritic findings, albeit with generally lower dF/F amplitudes (**Figure 20, Figure 21**). Comparing naive and trained states revealed significant enhancement of somatic responses in RL, particularly for tactile and visuotactile stimuli. In the general responder populations (**Figure 20**), RL tactile and visuotactile neurons showed marked potentiation post-training, while changes in V1 were less pronounced. This suggests that the learning-induced amplification of relevant sensory inputs is robustly transmitted to the neuron's output. Interestingly, when examining exclusively responding somata (**Figure 21**), we observed clear and substantial potentiation of the tactile response in RL neurons preferring tactile stimuli. These finding contrasts somewhat with the unclear dendritic changes in the small dominant tactile group (**Figure 19b**), a discrepancy that hints at complex soma-dendrite relationships during learning, a theme we explore further in the context of direct dendritic integration studies (see section 5.4).

Together, these somatic and dendritic data provide strong evidence that associative learning reshapes sensory representations in RL, dramatically amplifying responses to behaviorally relevant tactile and multisensory stimuli, consistent with functional

plasticity observed in cortical circuits during learning (Froemke et al., 2007; Gilbert & Sigman, 2007; Poort et al., 2015). This learning-induced enhancement, particularly in RL, provides a compelling cellular mechanism contributing to the improved behavioral performance and enhanced population signals observed during MSI, supporting and extending the findings of (Nabbefeld, 2022) by delving into the subcellular compartments involved.

1.4. Dendritic Mechanisms of MSI and Learning

A central goal of this thesis was to investigate MSI at the subcellular level, leveraging the computational power of dendrites. Inspired by studies such as Godenzini et al., which elegantly demonstrated the feasibility and importance of simultaneous somatic and dendritic recordings in L2/3 neurons to uncover compartmentalized computations and plasticity, we successfully established a robust methodology combining sparse labelling of L2/3 neurons using diluted Cre virus (**Figure 9**) with chronic, simultaneous 2Photon calcium imaging of somatic and apical dendritic activity in V1 and RL of awake, behaving mice (**Figure 13**) (Godenzini et al., 2021). The sparse labelling provided clear visualization of individual dendritic structures, and our initial recordings confirmed the feasibility of detecting distinct calcium transients in both compartments during sensory stimulation. This technical achievement paves the way for directly testing hypotheses about dendritic integration during MSI.

Our anatomical findings showing strong S1 input to superficial RL layers (L1/L2/3), where apical tufts reside, strongly suggest that these dendrites are prime candidates for implementing non-linear integration of visuotactile signals. The observed learning-induced enhancement of tactile responses in RL dendrites (**Figure 18**) fits well with this model, implying that S1 inputs to these apical dendrites are potentiated. Coincident visual input (potentially arriving via V1 into RL pathways targeting more proximal dendrites, or indirectly) and tactile input (via S1 into RL superficial projections) could trigger local dendritic events like NMDA spikes or Ca^{2+} spikes in the apical dendrites of RL neurons (Holtmaat & Svoboda, 2009; L. M. Palmer et al., 2014). Such events could lead to supra-linear summation of inputs, boosting the neuron's output and

contributing to the enhanced population responses and behavioral performance observed during multisensory trials. Godenzini et al. (2021), for instance, showed that pairing whisker stimuli could potentiate dendritic calcium responses in L2/3 of the barrel cortex neurons, highlighting the capacity of dendrites for input-specific plasticity (Godenzini et al., 2021). Our findings of tactile potentiation in RL dendrites are consistent with such a dendritic locus for learning. The ongoing analysis of the simultaneous soma/dendrite imaging data from the detection task (**Figure 14, Figure 17**) is expected to shed light on this possibility. While we hypothesized that multisensory stimuli would evoke significantly larger or more frequent dendritic calcium events in RL neurons compared to unisensory stimuli, particularly under conditions where behavioral enhancement is observed, our current population level analyses have not yet robustly demonstrated this specific dendritic signature of multisensory integration. This contrasts somewhat with the expectation that direct dendritic integration might yield strong supra-linear signals, though Godenzini et al. (2021) also noted that not all dendritic events strongly propagate to the soma under baseline conditions, suggesting complex integration rules (Godenzini et al., 2021). Several factors could contribute to this observation. It is possible that the magnitude of direct multisensory enhancement at the level of dendritic calcium signals is more subtle than anticipated at the population average, perhaps being present but not reaching strong statistical significance across all recorded neurons, comparable to some findings where multisensory effects can be modest though functionally relevant (e.g., (Meijer et al., 2020)). Alternatively, or in conjunction, the critical dendritic computations underlying successful multisensory integration might be more tightly linked to specific behavioral outcomes. Future analyses separating trials based on performance (i.e., correct vs. error trials) may be crucial to unmask such effects, as dendritic integration signals might be more pronounced or consistent only when the animal correctly utilizes both sensory cues to guide its decision. It is also conceivable that averaging across diverse neuronal responses or different phases of the task obscures more specific dendritic phenomena. Further refined analyses are therefore needed to fully elucidate the dendritic mechanisms of visuotactile integration in RL.

Furthermore, the longitudinal experimental design, incorporating passive exposure and active learning phases (**Figure 14d**), allows us to explore how dendritic processing evolves with experience. Our behavioral data from the detection task showed that while unisensory learning was gradual, multisensory performance was high immediately upon introduction (**Figure 14 b,c**). Analyzing the full dataset across all stages (Passive Naive vs. Passive Trained vs. Active Task) may reveal further modifications in dendritic excitability, input mapping, or the prevalence of dendritic spikes, contributing to improved sensory representations and task performance (Cichon & Gan, 2015; Gambino et al., 2014; Makino & Komiyama, 2015). These potential mechanisms find strong support in prior work; for instance, Cichon & Gan (2015) demonstrated that motor learning is associated with the formation of new dendritic spines and increased dendritic calcium activity, highlighting structural and functional dendritic adaptations crucial for memory encoding. Similarly, Gambino et al. (2014) provided direct evidence for the critical role of dendritic NMDA spikes in L2/3 pyramidal neurons of the barrel cortex during tactile associative learning, a finding highly pertinent to our study which also involves tactile learning in L2/3 and posits changes in dendritic excitability. Furthermore, Makino & Komiyama (2015) showed that learning induces branch-specific plasticity in dendrites of motor cortex neurons, illustrating how dendrites can selectively tune their responses and contribute to 'input mapping' and the refinement of representations. These studies collectively underscore the dynamic nature of dendrites during learning, providing precedents for the types of dendritic modifications, from structural changes to the generation of localized spikes and compartment-specific tuning, that could explain how unisensory inputs are refined and integrated within our associative learning paradigm. The observation that multisensory behavioral benefits appear rapidly (**Figure 14b, c**) while unisensory learning is gradual, coupled with our findings of significant plasticity in response to the unisensory components (especially tactile) after training, supports a model where the fundamental MSI circuitry might be largely pre-existing. This initial high multisensory performance, even when unisensory discrimination is still developing, can be interpreted through the lens of the principle of inverse effectiveness (Stein & Meredith, 1993). This principle posits that MSI benefits are often strongest for weaker or less

salient unisensory cues. While our psychometric curves (**Figure 14b, c**) primarily illustrate the learning trajectory of unisensory performance and the sustained high level of multisensory performance rather than parametrically testing inverse effectiveness across stimulus intensities, the initial weak or lower efficacy of the unisensory cues before extensive training could allow for more pronounced integration benefits when combined, thus explaining the immediate high multisensory success. Learning subsequently acts to gate or amplify these unisensory inputs (Meijer et al., 2020), effectively making them more salient components for the pre-existing integrative machinery. This improved salience of unisensory cues through learning is crucial for efficient processing, and the initial strong MSI might provide a scaffold or behavioral advantage while this unisensory tuning occurs.

The distinct learning-induced changes observed at the dendritic versus somatic level in some populations (e.g., the dominant tactile responders in RL, where somata showed clear potentiation (**Figure 21**) while dendrites showed fewer clear changes (**Figure 19b**), if these populations are indeed distinct or reflect different sampling) raises interesting questions about dendritic computation. While some research, notably from the Harnett lab, has suggested strong coupling and similar response properties between somatic and dendritic compartments in some contexts (Beaulieu-Laroche et al., 2019; Francioni et al., 2019), our results could be interpreted in several ways. Our findings, particularly the differing potentiation patterns in dominant tactile populations, resonate with the idea that dendritic and somatic compartments might not always show perfectly coupled plasticity or reflect identical computations, a concept explored by Godenzini et al. (2021) who demonstrated that dendritic calcium events, particularly those associated with NMDA spikes, can be localized and do not always lead to somatic action potentials, suggesting a degree of functional compartmentalization (Godenzini et al., 2021).

It is possible that learning induces distinct forms or magnitudes of plasticity in dendritic versus somatic compartments. For example, synaptic potentiation underlying the

enhanced tactile responses might occur predominantly on dendrites, leading to larger local dendritic signals. The degree to which this translates to somatic firing (and thus somatic calcium signals) could depend on additional factors like dendritic integration rules, local inhibition, or the overall excitability state of the neuron (G. J. Stuart & Spruston, 2015). If dendritic responses increase more substantially than somatic responses, or if different integration rules apply post learning, this could argue for a degree of compartmentalization in learning induced plasticity, not always resulting in a one to one somatic reflection. This is particularly relevant given that (Nabbefeld, 2022) reported multisensory enhancement in RL based on what was likely somatic or undifferentiated population activity; our work attempts to dissect whether these enhancements are uniformly expressed across compartments or if dendrites play a more specialized role in generating or shaping them.

Given that our analysis of dendrites and somata relied on imaging separate planes, and we were not longitudinally tracking the same identified ROIs across recording sessions, we cannot definitively link specific dendritic segments to their parent somata in these population-level comparisons. It is therefore possible that the dendritic planes captured a different subpopulation of neurons or dendritic branches than the somatic planes. This introduces the possibility of a sampling bias. The observed differences could then reflect distinct plasticity in these inadvertently separated samples. However, a key factor arguing against a complete dissociation of these populations is the targeted nature of our viral injections into deep layer 2/3. This approach makes it highly probable that the dendritic structures imaged in layer 1 predominantly originate from the somata transfected in layer 2/3, representing the apical tufts of these same neurons. Cortical regions, including L2/3, are known to harbor heterogeneous neuronal populations with diverse morphologies and input specificities (DeFelipe & Fariñas, 1992; Jiang et al., 2015). Furthermore, different dendritic compartments, such as distal apical tufts versus more proximal segments, receive distinct afferents and can exhibit specialized forms of plasticity (M. E. Larkum et al., 2009; Spruston, 2008). It is therefore conceivable that the dendritic imaging planes, optimized for capturing superficial L1 structures, preferentially sampled distal tuft segments that are major recipients of S1 input (as shown in our anatomical data, *Figure 8*) and may have undergone particularly strong

potentiation. Conversely, the somatic planes in L2/3 might have captured a population whose integrated output reflected a different net effect of plasticity, perhaps influenced by changes in other inputs or intrinsic properties not equally represented in the dendritic sample. The challenges inherent in optical imaging at different depths and the criteria for ROI selection could also contribute to sampling different effective populations over time if not tracking identical structures (Dombeck et al., 2010; Helmchen & Denk, 2005). Nevertheless, to definitively confirm the origin of the imaged dendrites and rigorously address this potential sampling bias, it would be preferable in future work to reconstruct the neurons within the imaged volume and beyond. Despite these considerations, the consistent overall trend of tactile response enhancement being prominent in RL in both compartments, at least when considering general responder populations (**Figure 18 and Figure 20**), does suggest a degree of coordinated plasticity across the neuronal elements within this associative area extending Nabbefeld findings on RL's adaptability to the subcellular level.

1.5. Limitations and Future Directions

While this thesis provides significant insights, several limitations should be acknowledged. Firstly, while we have presented data on passive sensory responses before and after learning, the full analysis of dendritic imaging data during active task engagement is currently incomplete. Final conclusions regarding the specific role of dendritic computation in MSI and learning, especially concerning non-linear integration during decision-making, await this comprehensive analysis.

Secondly, calcium imaging provides an indirect measure of neuronal activity and may not capture all aspects of electrical signaling, such as subthreshold events or precise spike timing (Grienberger & Konnerth, 2012). Correlating calcium signals with electrophysiological recordings in future experiments could provide a more complete picture.

Thirdly, our study focused primarily on L2/3 pyramidal neurons. While these superficial neurons play a crucial role in cortical processing and plasticity (M. Larkum, 2013; Petersen & Crochet, 2013), a complete understanding of the circuit dynamics requires investigating other cell types (e.g., inhibitory interneurons, crucial for shaping network activity) and, importantly, other cortical layers. A crucial future direction would be to directly compare the dynamics of visuotactile integration in L2/3 versus Layer 5 (L5).

L5 pyramidal neurons, in particular, represent a critical population that warrants investigation in the context of multisensory integration and decision-making. L5 neurons are the primary output neurons of the cortex, projecting to numerous subcortical targets involved in action selection and execution, such as the striatum, superior colliculus, and brainstem (Harris & Shepherd, 2015; Shepherd, 2004). This positions them as a key node for integrating processed sensory information to guide behavioral output. Furthermore, L5 neurons themselves receive a convergence of inputs, including feedforward sensory information, feedback from L2/3, thalamic inputs, and long-range cortico-cortical projections (Constantinople & Bruno, 2013; Hooks et al., 2011).

Crucially, substantial evidence from various cortical areas suggests that significant sensorimotor transformations and potentially complex multisensory interactions occur within L5. For example, studies have shown visual or auditory modulation of tactile responses in L5 of somatosensory cortex (Iurilli et al., 2012; Zagha et al., 2013), and activity in L5, particularly involving apical dendritic function, has been strongly linked to perceptual decision-making and motor control signals (Svoboda & Li, 2018; N. Takahashi et al., 2016). The distinct morphology of L5 pyramidal neurons, including their extensive apical tufts receiving input in L1 (similar to L2/3 but with different electrophysiological properties) and elaborate basal dendrites within L5, provides a substrate for unique integration computations potentially different from those in L2/3 (M. E. Larkum et al., 2009; Shai et al., 2015; H. Takahashi & Magee, 2009).

Therefore, it is plausible that the integration phenomena observed behaviorally, and potentially the supra-linear neural correlates seen in widefield imaging, might be strongly represented, or even primarily computed, within L5. A crucial future direction would be to directly compare the dynamics of visuotactile integration in L2/3 versus L5 pyramidal neurons within RL during the same evidence accumulation task. This could be achieved using layer-specific targeting strategies for GECI expression (e.g., specific Cre lines or targeted injections) or via multi-plane 2Photon imaging capable of resolving activity in both superficial and deep layers. Such comparative experiments would be essential to determine whether multisensory integration leading to enhanced perception is predominantly a function of superficial associative processing (L2/3), deeper output-related integration (L5), or a distributed process involving interactions across the cortical column.

Fourthly, the current work establishes correlations between neural activity, anatomy, and behavior. Future studies employing targeted manipulations (e.g., optogenetic or chemogenetic silencing/activation of specific pathways like S1 to RL or distinct dendritic compartments) are needed to establish causal links between the observed phenomena (Petreanu et al., 2009; N. Takahashi et al., 2016).

Based on the current findings and methodologies, several exciting future directions emerge:

The immediate priority is to complete the analysis of simultaneous somatic and dendritic imaging data during active task performance, explicitly testing for non-linear dendritic integration (e.g., supra- or sub-additive responses) by comparing multisensory responses to unisensory responses within identified soma-dendrite pairs, a technique refined in studies of dendritic function (Smith et al., 2013; Xu et al., 2012). This analysis will also involve correlating dendritic activity patterns with behavioral choices and learning stages, providing trial-by-trial insights into how subcellular processing contributes to perception, similar to approaches used to link dendritic activity to decision-making (N. Takahashi et al., 2016). The distinct learning-induced changes in the number (**Figure 22**) and amplitude (**Figure 18, Figure 19, Figure 20, and Figure**

21) of responsive units observed in passive conditions provide a crucial baseline for interpreting these task-specific modulations. To further investigate specific dendritic mechanisms, future work could combine calcium imaging with pharmacological manipulations, such as the local application of NMDA receptor antagonists or blockers of voltage-gated calcium channels, to directly probe the role of these specific conductances in multisensory integration, building on foundational work establishing their role in dendritic spikes and plasticity (M. E. Larkum et al., 1999; L. M. Palmer et al., 2014; Schiller et al., 2000). Utilizing genetically encoded voltage indicators (GEVIs) could also provide a more direct measure of electrical events in dendrites (Kannan et al., 2019). Causal manipulations, employing optogenetic or chemogenetic tools to selectively inhibit or activate specific pathways like the S1 into RL superficial projections or V1 into RL deeper projections, or even specific dendritic compartments using advanced optical methods (Mardinly et al., 2018; Yang et al., 2018), are essential to establish the necessity and sufficiency of these circuits and subcellular domains for the observed MSI benefits and plasticity. Such pathway-specific manipulations have proven powerful in dissecting circuit function in other contexts (Guo et al., 2014; Petreanu et al., 2009). Investigating cell-type specificity by combining calcium imaging with transgenic mice expressing Cre in distinct pyramidal subtypes or inhibitory interneurons (Pfeffer et al., 2013) will clarify the contribution of diverse neuronal populations to MSI. Exploring how dendritic integration adapts to varying task complexities (e.g., detection vs. discrimination) and stimulus parameters (e.g., testing the principle of inverse effectiveness by varying stimulus intensity) will provide a deeper understanding of the adaptive nature of dendritic computations. To confirm the micro-anatomical basis for dendritic integration, mapping the synaptic origins of S1 and V1 inputs onto RL dendrites using techniques like rabies tracing or GRASP (GFP Reconstitution Across Synaptic Partners) could determine if these inputs converge onto the same dendritic branches, a prerequisite for many forms of non-linear dendritic computation (Feinberg et al., 2008; Wickersham et al., 2007). Complementing imaging with in vivo patch-clamp electrophysiology will offer higher temporal resolution, allowing for the direct detection of local dendritic spikes and subthreshold synaptic events (Helmchen et al., 1999; Poulet & Petersen, 2008). Finally, integrating all

anatomical, physiological, and learning-induced functional data into computational models of the RL microcircuit will be crucial for simulating how laminar-specific inputs and dendritic non-linearities interact to generate the observed population activity and behavioral enhancements, providing a theoretical framework to test hypotheses about circuit function (Häusser & Mel, 2003).

1.6. Conclusion

All in all, this thesis provides a multi-faceted investigation into the neural basis of visuotactile integration and learning-induced plasticity in the mouse parietal cortex. We characterized the anatomical convergence of visual and somatosensory inputs onto RL, revealing distinct laminar targeting profiles providing a structural substrate for integration. Population-level recordings confirmed RL's role as an integration hub. Crucially, by comparing neuronal activity before and after associative learning, we demonstrated significant functional plasticity, particularly within RL. This plasticity manifested as both an *amplification* of somatic and dendritic responses to behaviorally relevant tactile and multisensory stimuli, and a sparsification and reorganization of the overall responsive neuronal populations (**Figure 18, Figure 19, Figure 20, Figure 21, and Figure 22**). This learning-induced enhancement and refinement provides a cellular mechanism for improved and more efficient sensory processing. Furthermore, we established the methodology for simultaneously imaging somatic and dendritic activity, paving the way for dissecting subcellular computations during behavior. Our findings strongly support the hypothesis that experience-dependent plasticity, potentially involving non-linear dendritic integration within RL L2/3 neurons facilitated by the dense superficial S1 input, is a key mechanism underlying the brain's ability to effectively combine and represent information across senses to guide perception and behavior.

Discussion

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Declaration of Authorship (Eidesstattliche Erklärung)

I, Sacha Abou Rachid, declare that this thesis and the work presented in it are my own and have been generated by me as the result of my own original research.

I do solemnly swear that /Hiermit erkläre ich an Eides statt:

1. This work was done wholly or mainly while in candidature for the doctoral degree at this faculty and university;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this university or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others or myself, this is always clearly attributed;
4. Where I have quoted from the work of others or myself, the source is always given. This thesis is entirely my own work, with the exception of such quotations;
5. I have acknowledged all major sources of assistance;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

Herby I state specific contributions of this thesis work, which will be later used for publication:

Sacha Abou Rachid and Bjoern Kampa designed and planned the experiments. Sacha Abou Rachid performed all experimental surgeries for viral injections, 2Photon

Eidesstattliche Erklärung

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imaging. Sacha Abou Rachid, Irene Lenzi, Simon Musall and Gerion Nabbefeld worked on the establishment of the behavioral setups used in this study. Behavioral training was performed by Sacha Abou Rachid and Svenja Mommertz. Sacha Abou Rachid performed 2Photon dendritic imaging experiments. Acquisition of microscopy data for the histological studies was performed by Sacha Abou Rachid. Anatomical analysis was performed by Sacha Abou Rachid. Thomas Rueland and Danique Holtrup worked on the 2Photon dendritic imaging analysis. Sacha Abou Rachid and Irene Lenzi performed the behavioral training and 2Photon somatic imaging. Gerion Nabbefeld worked on the 2Photon somatic analysis.

Date: 02/07/2026

Signature

A handwritten signature in black ink that reads "Sacha Abou Rachid". The signature is written in a cursive, flowing style with a large initial 'S'.