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## **Top-down regulation of visual processing and innate behaviors in the neonatal brain**

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*“Raphèl mai amècche zabì almi.”*

— Nimrod, *Inferno* XXXI, Dante Alighieri

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# 1 Introduction

## 1.1 General Principles of Early Cortical Development

Innate behaviors are essential for survival because they do not require prior experience or learning. In early life, such behaviors are thought to mainly depend on subcortical circuits and lower-order pathways, whereas higher-order cortical areas (HOAs) are assumed to mature too slowly to contribute much at this early stage. This view fits the classic model of cortical development, where primary sensory areas mature before association cortex and prefrontal areas [1–3]. Yet direct experimental evidence for this assumption is limited. The central question is therefore not only how neonatal circuits mature, but also whether HOAs can already influence sensory processing and innate behavior before sensory systems are fully mature.

To address this question, it is necessary to consider how early cortical circuits emerge and operate. Neonatal cortical circuits and activity do not resemble the continuous activity profile of the adult mature brain. Instead, they are dominated by intermittent bursts of highly correlated activity, separated by silent periods. These early patterns have been shown to serve a function, as they help shape circuit assembly, synaptic refinement, and the transition to sensory-driven processing [4–6]. Therefore, if HOAs contribute to neonatal behavior, they must do so within this immature and highly dynamic early neonatal network regime.

To understand how such early activity patterns emerge, and how HOAs may influence sensory processing at these early stages, it is necessary to first consider the developmental processes that establish the structural foundation of cortical circuits.

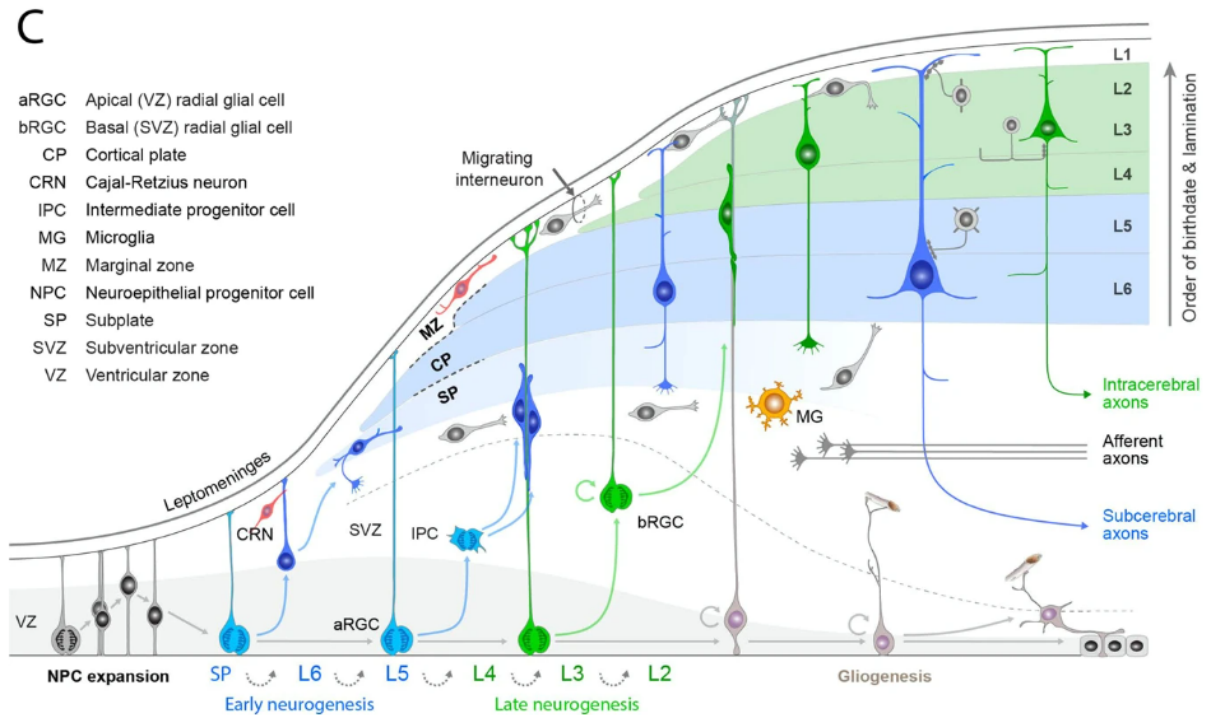
For most mammals, the development of neural circuits begins during embryogenesis, around gestation day (GD) 16 in humans and embryonic day (E) 7 in rodents, when neural progenitors (NPs) in the ventricular zone (VZ) start dividing symmetrically to expand the progenitor pool (Figure 1) [7]. At around E11.5 (~GD 25 in humans), NPs transition into radial glial cells, adopting an elongated morphology that enables them to serve both as the progenitors and the scaffolds for migrating neurons. At this point, asymmetric division begins, generating neurons that migrate along the scaffolding radial glial fibres to reach the developing cortical plate. The earliest born neurons initially form the preplate, which later splits into the marginal zone

and the subplate as new waves of neurons arrive. The cortical plate emerges between these layers, eventually developing cortical layers (L) 2-6. Neurogenesis follows a distinct inside-out pattern, where deep-layer projection neurons (L6 and L5) are born first, while later-born neurons migrate past them to form the superficial layers (L4, L3, and L2). As neurogenesis concludes (~E17.5), the radial glial scaffold supporting neural migration is dismantled, and neural progenitors shift towards gliogenesis, giving rise to astrocytes and ependymal cells [8]. This carefully orchestrated sequence of progenitor expansion, neurogenesis, neural migration and laminar organization establishes the structural foundation of functional neural circuits in the developing cortex before birth.

Once neurons have settled into their respective layers and begun forming synaptic connections, the next critical phase of cortical development involves the emergence of early activity patterns that drive network refinement. In the initial postnatal stages, cortical activity is primarily dominated by spontaneous intrinsically generated bursts that typically occur independently of sensory input. These transient bursts, characteristically separated by long periods of network silence, are a hallmark of immature neural circuits and are observed across most developing cortical networks [6,9,10]. During this initial postnatal phase, spontaneous activity plays a crucial role in shaping early neural circuits by regulating processes such as neurogenesis, neural migration, synaptogenesis, apoptosis and myelination [11,12]. As the sensory periphery matures, the transition from spontaneous to sensory-driven activity begins. Sensory input progressively modulates cortical activity, leading to more continuous and refined network dynamics. This shift ensures that cortical circuits become attuned to external stimuli, integrating sensory information to drive experience-dependent plasticity. The coordinated firing of neural ensembles during neonatal development has been detected across mammalian species as well as non-mammalian vertebrate and invertebrate species including zebrafish and the fruit fly [13,14]. This evolutionary conservation highlights the fundamental role of early activity in shaping sensory and cognitive function.

While these general principles broadly describe cortical development, different cortical areas follow distinct developmental trajectories. Lower-order areas (LOAs), such as the primary sensory cortices, and higher-order areas (HOAs), such as the prefrontal cortex, differ in their timing of maturation and functional development. The

following sections therefore describe the developmental trajectories of the primary visual cortex (V1), primary somatosensory cortex (S1), and the orbitofrontal cortex (OFC), with a particular emphasis on the prolonged and delayed maturation of HOAs.



**Figure 1: Illustration of early neocortical development.** Adapted Illustration from Kolk & Rakic, 2021 [7], originally from Silver et al., 2019 [15], illustrating the major developmental events and progenitors involved in the development of the cerebral neocortex.

## 1.2 V1 Development

The visual system has served as an ideal model system for identifying major transitions in cortical development and critical periods, owing to the accessibility of its pathway from the periphery to the cortex. The classic experiments by Hubel and Wiesel (1970) [16] pioneered the concept of critical developmental periods in the visual system. By suturing one eyelid of cats at different ages, they demonstrated that the effects of visual deprivation depend strongly on which developmental stage the cats were in. Monocular deprivation in adult cats with already fully developed vision, even for up to a year, had little to no effect. However, in younger cats only a few weeks old, even brief monocular deprivation for a couple of days caused permanent and severe deficits in visually evoked cortical responses [16]. These experiments established that visual experience

is essential during an early postnatal critical period, whereas the same manipulation in adulthood has no effect.

The rodent newborn is born completely blind, however, even before the onset of vision, neural activity is shaping the visual pathways. In neonatal rodents, during early postnatal development, postnatal day (P) 0-10, the eyelids remain closed shut, and rod/cone-mediated vision is almost entirely absent. However, the retina is still very much active and generates spontaneous transient bursts of action potentials, called retinal waves, which propagates across the retinal ganglion cells (RGCs) in a wave-like manner [17,18]. These waves are intrinsically generated, occur independently in each eye, are entirely independent of visual input and occur at a frequency of about once per minute, lasting around 3 seconds [19,20].

Interestingly, during this early period (P5—P8), when the mouse is functionally blind, a small percentage of the RGCs are highly specialized melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs), which already depolarize in response to light. These cells create a light-sensitive system separate from rods and cones, which can regulate non-image-forming visual functions, such as circadian rhythms, pupil responses, and even relatively complex intrinsic behavior such as light avoidance, where a mouse facing a direct light source will completely turn away from it [21]. Light avoidance has been observed as early as P6, before rod and cone-driven visual signalling. Moreover, ipRGCs have been shown to have reduced responses to retinal waves, providing a mechanism that makes them well-suited for light avoidance behavior, as it can function as a separate visual system, before the onset of image-forming vision [22].

Retinal waves play an important role in the development of visual processing and the visual system, as their development together with the early visual system progresses through three canonical developmental stages, each reflecting a different underlying mechanism that shapes retinotopic organization and connectivity with downstream targets (Figure 2) [23].

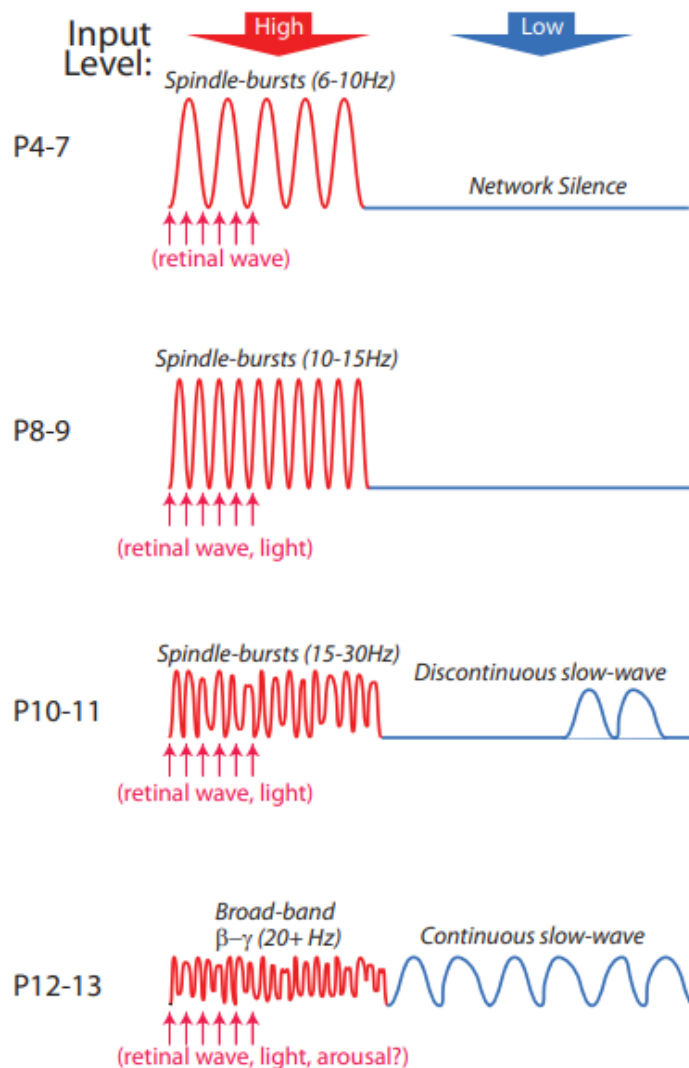
In stage 1 (late gestation), retinal waves are initially mediated by gap junctions and propagate as large and brief waves across the retina. This early phase plays a critical role in establishing coarse retinotopic maps in the SC and LGN [17,23,24].

In the second stage (P4-P10), as the retina gradually becomes light responsive, retinal circuits undergo a major transition. Here, synaptic transmission is largely

cholinergic, while GABA<sub>A</sub> signalling matures between P7 and P8, contributing to a gradual reduction in retinal wave size and speed, and an increase in their spatial precision [5,23,25,26]. At approximately P10, glutamatergic transmission gradually becomes the dominant excitatory input to RGCs, taking over from cholinergic signalling, causing changes in activity dynamics. Concurrently, during this stage, the retina gradually becomes light-sensitive, and RGCs begin transmitting neural signals to retinorecipient areas, including the SC, the LGN, and V1 [17,24].

In the third and final stage (P12 onward), retinal waves depend on glutamatergic input from bipolar cells to RGCs, which coincides with the first emergence of light responses in RGCs. At this stage, RGCs no longer rely on spontaneous activity from retinal waves but instead shift to continuous activity patterns driven by external visual input. This transition is essential for the maturation of visually evoked responses and the refinement of visual circuits. Disrupting activity during these stages impairs connectivity across the retina-LGN-V1 pathway [23].

After eye opening (~P12 in mice), retinal waves are completely replaced by continuous, visually evoked activity. At this point, RGCs no longer rely on spontaneous retinal waves, but instead convey patterned signals from the environment, enabling the maturation of orientation and binocular responses in V1 [5]. Thus, the development of the visual system proceeds through two stages: first through spontaneous intrinsic activity, independent of sensory input, and then through sensory-driven activity during critical periods. This sequence underlies the emergence of functional visual perception.



**Figure 2: Illustration of postnatal visual cortical development.** Adapted Illustration from Shen & Colonnese, 2026 [5]. The illustration depicts the transition of neuronal activity patterns in V1 across the first two postnatal weeks. Early ages are characterized by retinal waves and discontinuous, burst-like activity separated by periods of network silence, whereas later ages show a progressive emergence of continuous sensory-driven activity.

### 1.3 S1 Development

The primary somatosensory cortex (S1) in rodents is organized in an extraordinarily modular fashion, where each large whisker is represented in layer 4 by a discrete specialized cluster of neurons, forming so-called barrels. This discovery by Woolsey and Van der Loos (1970) [27] established S1 as a great model for linking cortical networks and architecture with peripheral input. Anatomically, these barrels arise from the clustering of thalamocortical axon terminals at the centre of each unit, with excitatory neurons and inhibitory interneurons forming a ring-like arrangement around these thalamocortical hubs. Functionally, this forms a one-to-one somatotopic map of the peripheral whisker pad, enabling processing of tactile information from individual



whiskers [28]. In classical deprivation studies, it was revealed that barrel formation depends critically on early whisker input. Removal of whisker follicles at birth prevents the corresponding barrels from forming [27], while trimming whiskers during the postnatal week alters the receptive field properties and weakens map precision [29,30]. These early experiments highlighted both the anatomical uniqueness and the developmental sensitivity of the rodent barrel cortex.

In neonatal rodents, however, whiskers are not yet used for active somatosensory exploration. Much like the visual system of newborn pups, the whisker system at birth is functionally immature. Instead, the neonatal activity profile in the developing barrel cortex is dominated by spontaneous bursting patterns, often driven by peripheral motor events such as twitches during sleep or self-generated whisker flicks [31–33]. These movements provide reafferent sensory feedback that reliably engages the thalamocortical circuits, producing structured bursts in S1. Like the developmental profile of the visual system, the developmental profile of S1 can also be best understood in stages or phases.

Based on a detailed analysis of spontaneous activity patterns in developing S1, Nakazawa et al. (2020) [34] proposed that the postnatal S1 develops and emerges functionally through successive activity phases, similar to what is observed in V1 development. In phase 1, starting around P1, patchwork-like spontaneous activity can already be observed within the developing S1, prior to barrel formation. From here and into the first postnatal week (P1-P7), thalamocortical input drastically starts to reorganize the L4, and start to form the barrel map. In this initial phase, both local and external inputs drive the development of S1.

In phase 2, typically around P9, layer 4 glutamatergic neurons start to show a strong spontaneous activity profile which is widely synchronized across multiple barrels. This creates an extraordinarily strong synchronous activity profile across the entire layer 4 of S1. This period, especially the transition from Phase 1 to Phase 2, is associated with a maturation of thalamocortical connectivity to the barrel map.

One of the most significant developmental steps takes place in phase 3, around P11. At this stage, transitioning between phase 2 and phase 3, the layer 4 neurons of S1 exhibit spontaneous activity that is much sparser and heterogeneously distributed in both space and time across the neuronal population. This type of organization is much more similar to that of the fully developed adult S1, as this type of activity sparseness

allows for precise and responsive information processing. Moving to around P14, mice will actively show exploratory whisking behavior, which again drastically increases the amount of information processed in S1 [34].

Together, studies on the visual and somatosensory systems illustrate a common developmental principle across lower-order cortical areas (LOAs). Both V1 and S1 undergo rapid and major structural and functional maturation during the first two postnatal weeks, where the systems are initially guided and dominated by transient bursts of spontaneous activity (retinal waves, spindle bursts), and later by the onset of sensory experience as the periphery matures. In both cases, early highly correlated activity gradually gives way to sparse decorrelated firing patterns, allowing for efficient sensory processing once the periphery is behaviorally engaged. Crucially, we see that this early period of sensory maturation is a critical period for later sensory processing and maturation, as any disruption at this stage has adverse effects for sensory function.

By contrast, higher-order cortical areas (HOAs) such as the prefrontal cortex mature much more slowly and along a less stereotyped trajectory. The following section will examine these developmental principles in HOAs, and how they diverge from the faster maturation of sensory regions such as V1 and S1.

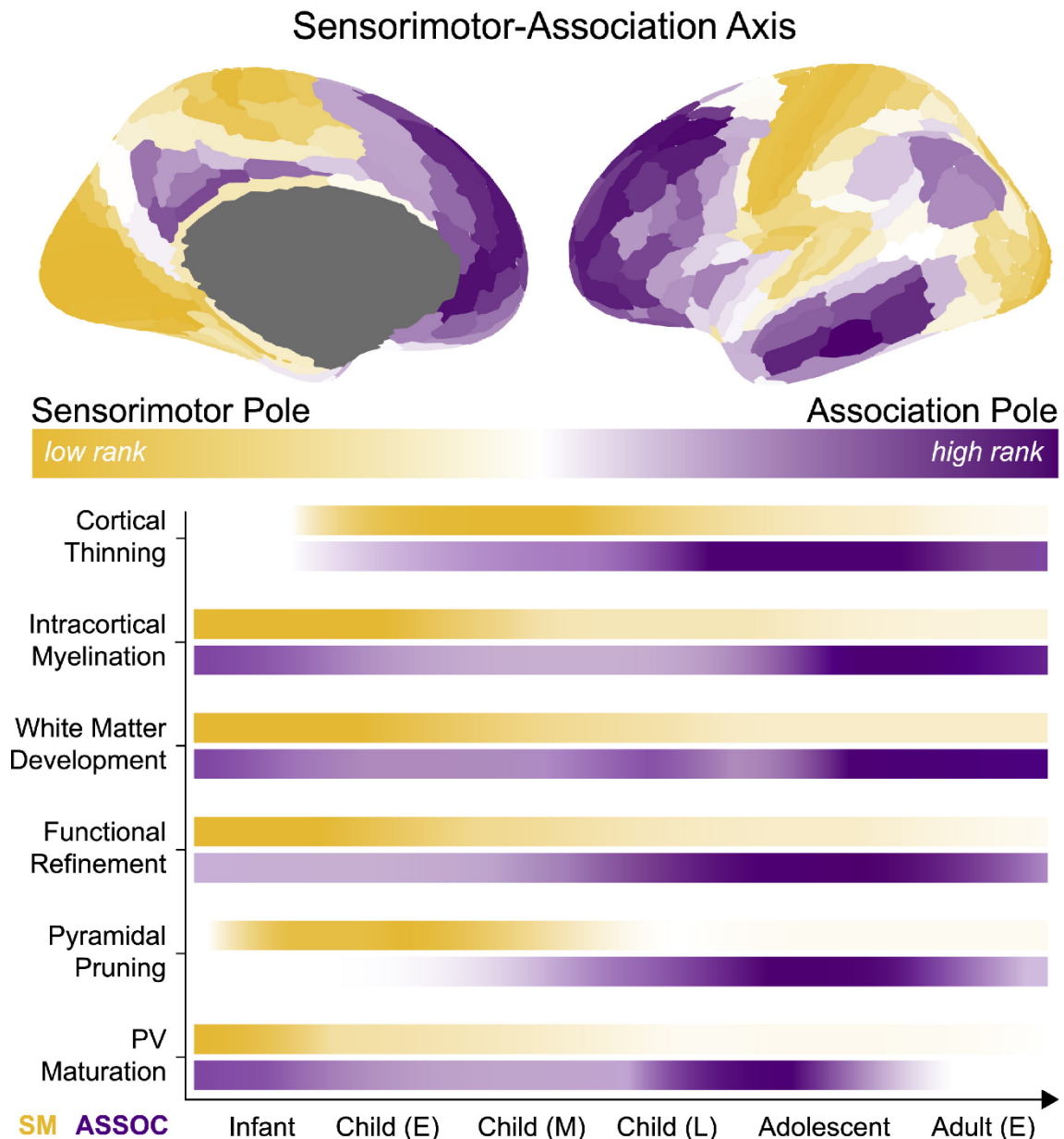
## **1.4 Higher-Order Cortical Areas: Protracted Maturation and Organization**

Early insights into the role of HOAs emerged primarily from lesion studies in humans and non-human primates. These studies revealed that damage to prefrontal regions caused profound impairments in behavioral flexibility, decision-making, and inhibitory control despite preserved sensory processing [35,36]. Whereas the function and development of the primary sensory cortices can be directly linked to peripheral input maturation and early activity patterns, HOAs were instead historically defined by their direct contribution to behavior rather than by identifiable developmental markers. As a consequence, understanding the cortical development of HOAs lagged behind that of LOAs, such as the sensory cortices, and was instead largely inferred from anatomical maturation processes including myelination, late synaptic pruning and the relatively late emergence of cognitive functions [37,38].

Compared to the relatively well-characterized developmental profiles of LOA, like V1 and S1, the maturation of HOAs, like the prefrontal cortex, remains

comparatively less defined [3]. Whereas LOAs are largely unimodal, functionally specific, and primarily involved in sensory processing, HOAs tend to be multimodal, functionally flexible, and central to information integration and higher-order cognition. HOAs contribute to a range of complex processes, including attention, salience detection, working memory, and inhibitory control, highlighting their role as adaptive and dynamic structures within the brain [35]. Importantly, such functions are generally recruited once an animal is already perceiving and interacting with its environment, reinforcing the concept of a developmental sequence across cortical regions.

A widely accepted principle in developmental neuroscience is that cortical maturation progresses along an occipital-to-frontal gradient, in which sensory regions mature first and prefrontal region later, thus HOAs develop over a more protracted period of time compared to LOAs (Figure 3) [37,39,40]. This gradient can be understood along three complementary axes: anatomical, functional and evolutionary. Examining each of these dimensions provides insight into why HOAs follow a distinct and protracted developmental trajectory compared to LOAs.



**Figure 3: Illustration showing the developmental gradients of lower- and higher-order cortical areas.** Adapted from Syndor et al., 2021 [39]. Illustration displaying the distinct developmental profiles across cortical hierarchies, with lower-order areas (yellow; sensorimotor pole) maturing earlier, and higher-order areas (violet; association pole) showing a delayed and more protracted maturation. These gradients are reflected across multiple developmental metrics, including myelination, functional refinement and pyramidal pruning.

### 1.4.1 Anatomical and Structural Frameworks

In the mature cortex, LOAs, such as V1 and S1, occupy the lower end of the anatomically defined cortical hierarchy and are characterized by high neuronal density and a predominance of feedforward cortico-cortical connections arising from supragranular layers, reflecting their primary role of further relaying sensory information from the sensory periphery [41,42].

By contrast, HOAs, including the prefrontal cortex, occupy the upper end of this cortical hierarchy and exhibit lower neuron density together with a greater proportion of feedback projections arising from infragranular layers. These hierarchical distinctions between LOAs and HOAs are further reflected in cortical lamination, as primary sensory cortical areas display a defined granular layer IV and dense thalamic input, whereas prefrontal regions lack a distinct layer IV and instead exhibit expanded supragranular layers that facilitate cortico-cortical feedback communication. The emergence of the anatomical distinctions between LOA and HOAs follows markedly different timescales. LOAs, such as V1 and S1, as we have seen, achieve adult-like topographical organization within the first two postnatal weeks, whereas HOAs undergo a far more protracted microstructural maturation in comparison, with many key anatomical features continuing to refine long into adolescence [40].

In the prefrontal cortex, for instance, synaptic density peaks substantially later than in V1, which attains stability following rapid synaptic pruning early during postnatal development [43]. Synaptic pruning in HOAs continues into adolescence, believed to be a key contributor to the prolonged structural plasticity observed in prefrontal areas [44]. Similarly, intracortical myelination is highest in the primary sensory cortices, but gradually declines from unimodal sensory regions to multimodal HOAs [45]. This delayed synaptic pruning and myelination in HOAs is associated with prolonged structural plasticity, which in turn extends the developmental window in which higher-order cortical connections can be refined [46].

A further key element of this anatomical gradient is the maturation of interneuron populations. During early postnatal development, somatostatin-expressing (SST) interneurons dominate the GABAergic landscape, as they are recruited relatively early into cortical networks on a similar timeline across cortical regions. Over the first postnatal weeks, however, SST input onto pyramidal neurons diminishes while parvalbumin-expressing (PV) interneurons increasingly target excitatory cells. In LOAs, PV circuits mature rapidly and are associated with the early stabilization of sensory networks; while in HOAs the integration of PV interneurons is markedly delayed, continuing into adolescence [47].

In parallel, inhibitory signalling undergoes a characteristic developmental transition in polarity. In the neonatal cortex, GABAergic transmission is initially depolarizing due to high intracellular chloride concentrations, only later towards the

second week of neonatal development does it become hyperpolarizing. This excitatory-to-inhibitory switch provides early network activity patterns that guide neural growth and synapse formation across cortical regions [48].

Together, these anatomical and cellular features establish distinct developmental constraints across the cortical hierarchy, providing a structural framework where functional differences between LOAs and HOAs emerge over time.

### **1.4.2 HOA Functional Maturation relative to LOAs**

From structure, function arises over development; and the microstructural features that distinguish LOAs and HOAs are mirrored in their functional roles and developmental timelines. LOAs, maturing early, transmit sensory input with high temporal precision and stability, supporting the rapid formation of stimulus maps and reliable perceptual representations. HOAs, by contrast, exhibit slower and more flexible dynamics, shaped by recurrent loops and multimodal integration. Their protracted maturation is associated with the later emergence of complex behaviors such as sustained attention, working memory, abstract reasoning, and inhibitory control; all functions that are only recruited once sensory cortices have stabilized [3,35].

A central determinant of cortical functional maturation during development is the gradual establishment of excitatory-inhibitory (E/I) balance, largely driven by the integration of interneurons into the cortical networks. In early postnatal development, the inhibitory circuits consist predominantly of SST interneurons, before PV interneurons are gradually integrated into the local cortical networks at later developmental stages [49,50]. In LOAs, this rapid PV integration together with the GABA polarity switch is often associated with the early transition from burst-dominated activity to a more decorrelated and sparse activity profile. In HOAs, delayed PV integration is associated with a more delayed inhibitory transition, prolonging periods of heightened plasticity and shifting the stabilization of higher cognitive functions to later developmental periods [3,51].

Taken together, LOAs stabilize quickly to support reliable sensory processing, whereas HOAs remain in a prolonged state of plasticity before higher cognitive functions consolidate. This developmental gradient is also mirrored on an evolutionary scale, where HOAs are disproportionally expanded in primates, something we will next

examine before returning to the prefrontal cortex as a concrete example of delayed HOA maturation.

### **1.4.3 Higher-Order Areas in the Evolutionary Cortical Hierarchy**

The anatomical and the functional hierarchies can be mapped onto an evolutionary cortical hierarchy. All mammals possess primary sensory areas, which all share many features of thalamocortical and corticocortical connections, and these regions likely dominated the cortical organization in the earliest mammalian brains. Across species, primary sensory regions expanded and underwent functional specialization [52]. In higher primates, however, the cortical surface dedicated to sensory and motor regions declined proportionally, whereas the cortical areas dedicated to HOAs expanded [53]. Among these, the prefrontal cortex expanded most dramatically, with humans showing a far greater prefrontal enlargement than macaques and chimpanzees [54].

From an evolutionary perspective, HOAs occupy the upper end of the cortical hierarchy, while phylogenetically older cortical regions like LOAs remain at the base. This evolutionary trajectory is not only associated with an increase in the cortical real estate dedicated to higher-order cognitive processing, such as multimodal integration, but it is also linked to the extended developmental timeline of HOAs. This, in turn, reinforces the gradient from early maturing evolutionary conserved LOAs to late-developing, evolutionary recent HOAs.

These evolutionary differences raise questions about prefrontal homology across species, particularly between humans and rodents. The common ancestor of modern humans and mice diverged about 96 million years ago [55]. In primates, the prefrontal cortex is homotypical, with six cortical layers including a granular layer IV, an organization that supports a dorsolateral PFC specialization that is absent in rodents. The rodent prefrontal areas are agranular, lacking a granular layer and a clear dorsolateral homolog [35], leading some to question the translational relevance of rodent PFC in understanding primate PFC function [56].

Nevertheless, a broad consensus argues for functional homology between rodent and human prefrontal cortex. What the PFC does, rather than its exact cytoarchitecture, can still be conserved [57,58]. While there are clear structural differences between humans and the rodent prefrontal cortex, including species-specific structures like the dorsolateral PFC in humans, rodents could hold the

functions related to this region in other areas of the PFC, like the medial PFC or orbitofrontal cortex (OFC) [59]. Indeed, lesion studies reveal shared functional deficits across species, supporting the idea that core prefrontal functions are class-common despite anatomical divergence [60]. Thus, rodent HOAs remain invaluable for dissecting the mechanisms and development of cortical areas, even as certain anatomical specializations are primate-specific. Yet despite this framework, the specific developmental profile of the prefrontal cortex itself remains patchy and less well defined, and only recently have studies begun to unveil its development.

## **1.5 The Prefrontal Cortex: Developmental Trajectories**

While the anatomical, functional, and evolutionary gradients provide a framework for understanding why HOAs follow a protracted developmental trajectory compared to LOAs, a precise developmental profile of the prefrontal cortex itself is largely missing. Characterizing prefrontal development requires focusing on the activity patterns that emerge early during neonatal development, and how they mature over time.

At the network level, like sensory cortices, neonatal PFC activity is dominated by periods of complete network silence, punctuated by transient bursts of coordinated activity. A hallmark of early prefrontal activity dynamics is the presence of beta-low-gamma oscillatory bouts [61,62], which represent a characteristic developmental activity motif. Perturbations of these oscillations during early postnatal development have been shown to interfere with the later-emerging cognitive performance [63].

The origin and early shaping of these oscillatory bouts are thought to involve pyramidal neurons in the supragranular layers of the PFC and their interactions with afferent inputs. Prefrontal oscillations occur naturally in response to hippocampal drive, and optogenetic activation of pyramidal neurons in intermediate CA1 has been shown to boost prefrontal oscillatory activity during neonatal development [64]. Over development, as prefrontal oscillatory activity gradually emerges and evolves, they become longer-lasting, faster, and higher in amplitude from the first postnatal week into adulthood [65].

While hippocampal input is a prominent driver of neonatal prefrontal rhythmic activity, the emergence of adult-like activity dynamics depends on the concurrent maturation of local inhibitory circuits. As in other cortical regions, neonatal PFC is initially dominated by SST activity. Over development, synaptic strength from SST



interneurons onto pyramidal cells declines, while PV interneurons are recruited and progressively strengthen their connectivity. Whereas adult-like fast ( $>30$  Hz) oscillations emerge already in the second postnatal week in LOAs and in the hippocampus in mice, fast oscillations in HOAs such as the medial PFC matures substantially later, reaching stability only by the end of postnatal week four [63,66,67]. This transition from SST- to PV-dominated inhibition has been implicated in the ontogeny of prefrontal gamma oscillations and the later emergence of interhemispheric synchrony in the developing PFC [68].

Crucially, early prefrontal activity does not emerge in isolation. Whether activity patterns generated within the developing PFC convey information to downstream targets remains an open question. What is clear, however, is that the functional maturation of the PFC relies on other cortical and subcortical regions, including the ventral tegmental area, striatum and the hippocampus. The hippocampus critically contributes to the functional development and maturation of prefrontal networks [64]. The most prominent activity patterns that dominate the developing hippocampus are sharp wave ripples. The CA1 of the hippocampus accounts for a major glutamatergic input in the developing PFC, and sharp wave ripples induce a prominent increase in prefrontal firing rate and a broadband increase in local field potential (LFP) power. CA1 inputs to the PFC have been shown to promote and increase intracortical coupling and the emergence of prefrontal oscillatory rhythmic activity [69].

As prefrontal circuits mature and stabilize, their role progressively shifts from being driven by distributed inputs to exerting top-down influence over downstream cortical regions. Among prefrontal subregions, the orbitofrontal cortex (OFC) is particularly well positioned to mediate such top-down modulation of sensory processing and behavior.

## **1.6 The Orbitofrontal Cortex, Top-down Modulation and Innate Behavior**

One of the defining functions of the mature PFC is to exert top-down influence over LOAs, such as the sensory cortices. The PFC is interconnected with visual areas in the cortex, which puts it in a suitable position to influence visual representations in line with attentional demands [70]. Prefrontal top-down projections to sensory cortices have been shown to directly modulate sensory processing by affecting accurate sensory

perception, predictive signalling, and promoting adaptive behavior [71,72]. These projections are also central for cognitive control, allowing context, internal states, and goals to shape sensory information. However, it remains largely unknown when such top-down control emerges during development. In contrast to the relatively well-defined developmental profiles of LOAs, very few studies have examined the timing, mechanisms, or the functional impact of prefrontal feedback on developing immature LOAs. It remains unknown whether such prefrontal influences are present during early neonatal development or whether they only emerge later in adolescence. Clarifying when top-down influences emerge is essential for understanding how HOAs integrate with early sensory networks and shape behavior before learning.

Among the prefrontal HOAs, the orbitofrontal cortex (OFC) stands out as a conserved hub for multisensory processing. Anatomical tracing studies have established OFC as a highly interconnected brain region in the adult mouse, forming dense projections to multiple LOAs, including primary sensory areas [73,74]. In the adult mouse, direct long-range projections from the OFC to V1 and S1 have been mapped, and large-scale connectomics rank OFC among the strongest afferent projecting areas, surpassed only by thalamus and the anterior cingulate [75–77]. Importantly, OFC projections to V1 show a high degree of cell-type specificity, with axons preferentially targeting SST interneurons over other subtypes [78]. This selective connectivity highlights OFC as a prominent source of top-down input to the visual system. Whether and when these anatomically defined top-down pathways are present and functionally active during neonatal development, however, remains unknown.

Much of our understanding of OFC function stems from studying the effects of structural brain damage in humans. Patients with ventromedial/orbitofrontal cortex damage exhibit impaired decision-making, fail to adjust behavior based on changing environmental contingencies and feedback, and perform poorly in tasks that depend on such processes, like gambling paradigms [79]. These deficits demonstrated a critical role for the OFC in adapting behavior in response to environmental change and feedback. This helped establish the OFC as a critical structure for adaptive, outcome-guided behavior. Subsequent work has shown that OFC activity encodes the subjective value of anticipated outcomes and signals when actual outcomes deviate from expectations [80,81]. In this way, the OFC supports flexible updating of behavior

in dynamic environments by continuously integrating new contextual information, expected value, and sensory input.

Animal studies have confirmed that these functions are conserved across species. In both rodents and primates, OFC neurons dynamically adjust their activity according to changing contingencies, and lesions or activity inhibition impairs the ability to reverse learned associations [82,83]. Such reversal learning paradigms are widely used behavioral assays in targeting OFC function, as they directly probe OFC's role in updating outcome expectations and remapping behavioral strategies when environmental rules change. Beyond behavior, experimental manipulations have demonstrated that OFC activity can reshape sensory representations. For example, optogenetic activation of the OFC suppresses visually evoked responses in V1. This inhibitory effect is thought to arise from recruitment of local SST interneurons in V1 [78]. Similarly, silencing OFC-S1 projections disrupts sensory plasticity and impairs reversal learning [77].

Taken together, this body of evidence highlights the OFC as a hub for flexible, value-based control of both behavior and sensory processing. By integrating outcome evaluation with direct feedback to primary sensory cortices such as V1, the OFC occupies a central position in regulating how animals adapt to shifting contexts and unexpected events. The OFC is therefore well positioned to influence early sensory processing, raising the possibility that top-down signals could already modulate innate behaviors that do not depend on prior learning.

Despite its central role in mature and adult cognition and complex behavior, very little is known about how OFC-driven top-down signals influence behavior during early life. One reason for this gap is the difficulty in probing complex behavior in neonatal animals, which at this stage have a restricted behavioral repertoire. The first context in which top-down influences could plausibly manifest during early life is therefore through innate behavior. Such behaviors are particularly suited for developmental studies as they do not depend on prior learning and can therefore potentially reveal the earliest functional impact of top-down modulation. This link between OFC development and innate behavior forms the basis of the present work.

### **1.6.1 Early Light-Avoidance as a Model for Innate Behavior Control**

Innate behaviors are evolutionarily conserved, stereotyped actions that emerge without prior learning, and are essential for survival in animals [84]. They provide the animal with immediate, highly adaptive responses to certain stimuli, such as orienting towards or away from light, withdrawing from potentially harmful stimuli, or approach/avoid tendencies. While many of the innate defensive responses are highly conserved across species, ranging from fruit flies to mammals, most of the responses do not emerge until later in development [85,86]. However, some visually evoked innate defensive responses have been shown to be present early in neonatal development, and a striking example of this is light avoidance in neonatal mice. As introduced in the context of V1 development, light avoidance emerges as early as P6 in neonatal mice via ipRGC signalling, before the onset of image-forming vision [21,22].

Such innate behaviors are traditionally thought to largely rely on LOAs [87–89], if not entirely on subcortical circuits that bypass sensory cortices [6,90]. In contrast, HOAs are thought to refine, contextualize, or flexibly adapt such instinctive behaviors [4,91]. Considering HOAs have a protracted development, their contribution to innate behavior early in life has been postulated to be even more limited, yet experimental evidence is missing. As such, whether innate behaviors like light avoidance are exclusively orchestrated from peripheral and subcortical processing or whether it depends on HOAs during neonatal development is unclear. Although innate behaviors span multiple sensory modalities, we focus here on a visually evoked response since anatomical mapping revealed stronger projections from OFC to V1 than to S1 during early development, suggesting that the connectivity between HOAs and LOAs may not be uniform across sensory modalities. Here, we consider light avoidance not as a purely retinal phenomenon but as an innate behavioral readout, through which the earliest potential top-down influence from the OFC can be investigated.

## **1.7 Objectives**

The aim of this thesis is to determine how and when higher-order cortical activity engages developing sensory networks during early postnatal life. To address this, we characterize the developmental activity profiles of OFC and primary sensory cortices, map their anatomical connectivity, and assess the functional effects of OFC activity on sensory processing and innate behavior during early development.

1. Characterize and compare the developmental activity profiles of the OFC, V1 and S1 during early neonatal development
2. Map monosynaptic projections between OFC and primary sensory cortices and determine their predominant directionality during neonatal development.
3. Assess how optogenetic activation of OFC influences visual processing in V1 during neonatal development.
4. Test whether OFC-driven modulation of V1 activity during neonatal development engages SST interneurons.
5. Test whether chemogenetic inhibition of OFC alters visually driven innate light avoidance during neonatal development.

## 2 Material and methods

### 2.1 Material

**Table 1. Material**

| REAGENT OR RESOURCE                    | SOURCE            |
|----------------------------------------|-------------------|
| <b>Chemicals, tracers, and viruses</b> |                   |
| Isoflurane                             | Baxter            |
| Ketamine                               | Richter Pharma AG |
| Xylazine                               | Bayer             |
| Fluoromount                            | Thermo Fisher     |
| Lidocaine                              | UKE               |
| Bupivacaine                            | UKE               |
| Omnifill C                             | Omni Dent         |
| Compound 21                            | Hello Bio         |
| Paraformaldehyde                       | Carl Roth         |
| DAPI                                   | Sigma-Aldrich     |
| Fluorogold                             | Fluorochrome, LLC |
| AAV9-CaMKII-hM4D(Gi)-mCherry           | Addgene           |
| AAV9-Ef1a-DIO-hChR2(E123T/T159C)-EYFP  | Addgene           |
| AAV9-CaMKII-hChR2(H134R)-mCherry       | Addgene           |
| <b>Experimental Model</b>              |                   |
| Mouse: C57BL/6J (Black 6)              | UKE               |
| Mouse: SST-IRES-cre                    | UKE, ZMNH         |
| <b>Software and programs</b>           |                   |
| ImageJ 2.0.0                           | ImageJ            |
| MATLAB                                 | MathWorks         |
| Adobe Illustrator                      | Adobe             |
| R Statistical Software 4.2.2           | RStudio           |
| Cheetah 6                              | Neuralynx         |
| Phy                                    | Cortex-Lab        |

|                                                                           |                                |
|---------------------------------------------------------------------------|--------------------------------|
| Klusta                                                                    | kiwikteam                      |
| Python 3.8                                                                | Python                         |
| Cellpose                                                                  | Stringer Lab                   |
| Arduino Uno                                                               | Arduino                        |
| Anaconda 1.9.12                                                           | Anaconda, Inc                  |
| <b>Other</b>                                                              |                                |
| Diode laser (473nm)                                                       | Omicron                        |
| Recording electrode<br>(1 shank, 16 channels, 50µm<br>inter-site spacing) | Neuronexus                     |
| Recording electrode<br>(4 shank, 16 channels, 50µm<br>inter-site spacing) | Neuronexus                     |
| Recording optrode<br>(1 shank, 16 channels, 50µm<br>inter-site spacing)   | Neuronexus                     |
| Recording optrode<br>(4 shank, 16 channels, 50µm<br>inter-site spacing)   | Neuronexus                     |
| Optic patch cable                                                         | Thorlabs                       |
| Optical Power Meter                                                       | Thorlabs                       |
| Microinjection and pump<br>controller                                     | World Precision<br>Instruments |
| Nanofil Microsyringe 10 µl                                                | World Precision<br>Instruments |
| NanoFil Needle 34G                                                        | World Precision<br>Instruments |

## **2.2 Method**

### **2.2.1 Animals**

All experiments were approved by the University Medical Center Hamburg-Eppendorf guidelines and the institutional animal welfare officer. All procedures were performed in accordance with German Animal welfare Act and were approved by the State Authority of Hamburg (Behörde für Justiz und Verbraucherschutz, Amt für Verbraucherschutz, Lebensmittelsicherheit und Veterinärwesen, Germany (N18/015, N19/121). Timed pregnant mouse dams were housed either individually or in groups of two at a 12 h light/12 h dark cycle and were given access to water and food ad libitum at a room temperature of 21 °C and humidity at 43%. The day of birth was considered P0. Experiments were carried out on C57BL/6J and on SST-Cre (SST-IRES-cre) mice of both sexes, at the age of P5-12. Each animal underwent a single acute recording session.

### **2.2.2 Fluorogold injections**

P8/P9 pups were placed in a stereotactic apparatus and kept under anesthesia with isoflurane (induction level: 5%, maintenance level: 1-3%) for the entire procedure. For retrograde tracing, fluorogold (FG; 2.5%, Fluorochrome, LLC) was iontophoretically injected into OFC (1.5 mm anterior to bregma, 0.5-0.8 mm lateral to the midline) or V1 (3 mm posterior to bregma, 1.5-2 mm lateral to the midline) of P8/P9 mice. A glass capillary (~ 25 µm tip diameter) was filled with ~ 1 µl FG by capillary forces, and a silver wire was inserted such that it was in contact with the FG solution. The positive pole of the iontophoresis device was attached to the silver wire, whereas the negative pole was attached to the mouse body (skin of the neck or the tail). The capillary was carefully lowered into the OFC (~ 2mm dorsal from the dura), V1 (~ 0.3mm dorsal from the dura) or S1 (~ 0.3mm dorsal from the dura). For injections, an anodal current to the capillary (6 s on/off current pulses of 6 mA) was applied for 10 min. Post injection, the capillary was left in the injection site for an additional 2-3 minutes to avoid reflux before slowly being withdrawn. After injections, pups were placed on a heating blanket (37 °C) until full recovery before being returned to the dam. The pups were perfused 3 d after injection for assessment of FG expression.



### **2.2.3 Virus injections**

P1-P3 pups were anesthetized with isoflurane (3 %) and fixed into a stereotaxic apparatus and AAV9-CaMKII-hChR2(H134R)-mCherry (Plasmid #26975, Addgene; 100 nL in right hemisphere) was injected unilaterally into the OFC at a rate of 50 nL/min using a syringe infusion microinjector (NANOFIL 34G, WPI). Following the injections, the syringe was left in the injection site for an additional 2-3 minutes to avoid reflux of the fluid before slowly being withdrawn. After injections, pups were placed on a heating blanket (37 °C) until full recovery before being returned to the dam. Expression of ChR2-mCherry was detected using a dual fluorescent protein flashlight (Electron Microscopy Sciences) before surgery and confirmed post-mortem. Only mice with sufficient expression of fluorescence were included in subsequent experiments.

For phototagging experiments, P1-P3 SST-Cre positive pups were injected with AAV9-Ef1a-DIO-hChR2(E123T/T159C)-EYFP (Plasmid #35509, Addgene; 100 nL in right hemisphere) into V1 (3 mm posterior to bregma, 1.5-2 mm lateral to the midline, and ~ 0.3mm dorsal) following the same setup and protocol.

For chemogenetic inhibition of OFC using DREADDs, P1-P3 pups were injected bilaterally with AAV9-CaMKIIa-hM4D(Gi)-mCherry (Plasmid #50477, Addgene; 80 nL in each hemisphere) in OFC (1.5 mm anterior to bregma, 0.5-0.8 mm lateral to the midline, ~1.8-2 mm dorsal from the dura) following the same setup and protocol. To activate DREADDs, we injected Compound 21 (C21, Hello Bio, 0.3mg/mL<sup>-1</sup> dissolved in 0.9% NaCl) intraperitoneally (i.p.) at P11 or at P12.

### **2.2.4 Acute multi-site electrophysiological recordings**

Multisite extracellular records were performed unilaterally (right hemisphere) in the OFC, V1, and S1 of non-anesthetized P5-12 mice. The animals were removed from their home cages only at the time of the surgical procedure. The surgical procedure was carried out under isoflurane anesthesia (induction level: 5%; maintenance level: 1-3%, higher levels for younger pups and lower levels for older pups) on a heated surface (37°C). A local anesthetic was applied to the neck muscles of the mice (0.5% bupivacaine / 1% lidocaine) before they were cut to reduce muscle artefacts. A craniotomy over the OFC (1.5 mm anterior to bregma, 0.5-0.8 mm lateral to the midline), V1 (3 mm posterior to bregma, 1.5-2 mm lateral to the midline), S1 (1 mm

posterior to bregma, 2.5-3 mm lateral to the midline), and cerebellum was performed by carefully thinning the skull and then removing it using a motorized drill.

Mice were then head-fixed into a stereotactic frame and kept on a heated (37°C) surface for the entire recording. Electrodes/Optrodes were carefully inserted into OFC (four-shank, 4x4 recording sites, 100 µm inter-site spacing; depth 2-2.2mm), and V1 and S1 (one-shank, 1x16 recording sites, 50 µm inter-site spacing; depth 0.8mm). Before insertion, the electrodes were covered with Dil to enable the construction of electrode position post-mortem in brain slices. A silver wire was implanted between skull and brain tissue above the cerebellum and served as ground and signal reference. Mice were allowed to recover from surgery for 20 min before the recording procedures were initiated.

### **2.2.5 Signal acquisition**

Extracellular signals were acquired and digitized at a 32 kHz sampling rate after band-pass filtering (0.1-9000 Hz) using an extracellular amplifier (Digital Lynx SX; Neuralynx, Bozeman, MO, Cheetah, Neuralynx, Bozeman, MO). The length of the individual baseline recording session varied between 30 and 120 minutes.

### **2.2.6 Optogenetic and retinal stimulation**

Optogenetic stimulation was performed using an Arduino Uno (Arduino, Italy) controlled laser system (473 nm wavelength, Omicron, Austria) coupled to a 105 µm diameter light fiber (Thorslabs, NJ, USA) attached to one of the optrodes, ending 200 µm above the top recording site. Ramp (linearly increased light power) light stimulation and pulsed (short pulses of 3 – 50 ms, depending on the experimental paradigm) light stimulation at different frequencies were delivered. At the beginning of every recording session, the response to light stimulation was tested using 3 ms pulses at 16 HZ frequency, and only reliably responding animals were recorded. Laser power was titrated before signal acquisition and adjusted to the minimum level that induced the desired neuronal response.

External retinal light stimulation was performed using the same laser setup as for the optogenetic stimulation paradigm, but with the optic fiber directed directly at the left eye (contralateral to the recorded right hemisphere) of the mouse. Light stimulation was delivered either as pulses or as ramp stimulations, depending on the experimental

paradigm. Laser power was increased until a consistent visual response was seen in V1 activity.

### **2.2.7 Opto-tagging**

ChR2-EYFP expressing SST interneurons were stimulated with 100 sweeps of 3 ms light pulses at 473 nm wavelength delivered through an optrode. Firing rate increase at 10 ms after stimulation onset (firing rate<sub>stim</sub>) was compared to a pre-stimulus window of the same length (firing rate<sub>pre</sub>). Significantly activated units were defined as  $p < 0.001$  (Wilcoxon signed-rank test) and  $MI > 0.2$  ( $MI = [\text{firing rate}_{\text{stim}} - \text{firing rate}_{\text{pre}}] / [\text{firing rate}_{\text{stim}} + \text{firing rate}_{\text{pre}}]$ ).

### **2.2.8 Histology**

Mice (P5-12) were deeply anesthetized with 10% ketamine/2% xylazine in 0.9% NaCl (10 mg/g body weight, intraperitoneal injection) and transcardially perfused with 4% paraformaldehyde. Brains were removed, postfixed in 4% paraformaldehyde for 24 h, and coronally sectioned with a vibratome at 100  $\mu\text{m}$  thickness. Slices were mounted with Fluoromount (Thermo Fisher Scientific) + DAPI (Sigma-Aldrich) mounting medium. The opsin expression and Fluorogold expression, as well as the position of electrodes in OFC, V1, and S1, were all assessed using epifluorescence microscopy.

### **2.2.9 Cell quantification**

High-resolution single images were captured using a fluorescence binocular microscope with a resolution of 5760 x 3600 pixels. Image acquisition was performed using a 568 nm laser for visualizing mCherry expression, a 488 nm laser for visualizing EYFP expression, and ultraviolet (UV) light for visualizing fluorogold expression. For the fluorogold cell quantification, cellular detection was performed using Cellpose [92], a deep learning-based segmentation algorithm implemented in Python 3.8. The detection parameters remained constant for all images, and the validity and accuracy of the counted result were verified through visual inspection.

### **2.2.10 Negative optotaxis assay**

Light avoidance in neonatal mice was tested using a similar behavior setup and protocol as previously described [21]. The behavioral setup consists of a rectangular open chamber placed on a heated mat. The behavioral assay spanned two sessions

on two following days. For each session on one of the given days, shortly before testing began, pups P11-P12 were either injected with Compound 21 or with saline. Pups' movements were recorded in the open chamber with a video camera. The position of the pup was monitored for a 2-minute period, followed by a 2-minute period with bright light directed at the face of the pup. Responses to light were measured by the latency at which the mouse first made a turn away from the light. All tests were performed at room temperature.

### **2.2.11 Negative geotaxis motor function assay**

To assess motor function and motor reflexes, a subset of mice that underwent the negative optotaxis assay had their negative geotaxis reflex tested to assess general motor function. The test and behavioral setup were made as previously described [21]. The pups were placed with their head pointing downwards on a  $\sim 45^\circ$  inclining slope and held in that position for approximately 5 seconds. The pups were followingly released, and the time it took the pup to turn and face upwards was recorded with a video camera. The total testing time was 2 minutes. Mice that fell or failed to turn were retested a maximum of two times.

### **2.2.12 Data Analysis**

Data were analyzed using custom-written MATLAB (<https://github.com/OpatzLab/HanganuOpatzToolbox>) and Python scripts.

### **2.2.13 Power spectral density**

LFP signals at a sampling frequency of 32 kHz were acquired from 16 channels. The signal was band-pass filtered in the range of 1-500 Hz using a third-order Butterworth filter, applied in a phase-preserving manner to ensure the integrity of signal timing. The data was then downsampled by a factor of 32. The LFP signals were re-referenced to the mean of all channels. The power spectrum density (PSD) was computed using Welch's method, with overlapping windows (window size: 1 second, overlap: 250 ms).

### **2.2.14 Spectral Dependence Ratio**

The spectral dependence ratio (SDR) was used to assess the directional relationship between the PSDs of two signals, as previously described in similar methods [93,94]. The SDR quantifies the interaction between frequency bands of two signals by

comparing their spectral power across defined frequency ranges. The SDR was calculated using the following formula:

$$SDR_1 = \frac{\text{mean}(PSD_2(F))}{\text{mean}(PSD_1(F)) \cdot \text{mean}\left(\frac{PSD_2(F)}{PSD_1(F)}\right)}$$

$$SDR_2 = \frac{\text{mean}(PSD_1(F))}{\text{mean}(PSD_2(F)) \cdot \text{mean}\left(\frac{PSD_1(F)}{PSD_2(F)}\right)}$$

$F$  represents the specified frequencies for analysis. If no frequency bins were specified, the SDR was calculated using the entire frequency range, comparing the overall mean power between both signals.

### 2.2.15 Single-unit activity

Spike sorting was performed using Klusta (<https://github.com/kwikteam/klusta>) and automatically detected clusters were then manually curated using Phy (<https://github.com/cortex-lab/phy>).

### 2.2.16 Spike time tiling coefficient

The STTC, a measure of the interaction strength between spike trains that is robust to changes in firing rate, was calculated as previously described [95] (Figure 3A):

$$STTC = \frac{1}{2} \left( \frac{P_A - T_B}{1 - P_A T_B} + \frac{P_B - T_A}{1 - P_B T_A} \right)$$

where  $P_A$  is the proportion of spikes of spike train A that occur within  $\pm\Delta t$  of a spike train B spike.  $T_A$  is the proportion of time that occurs within (is “tiled” by)  $\pm\Delta t$  from spikes of spike train A. The same applies for  $P_B$  and  $T_B$ .  $\pm\Delta t$  is the lag parameter, as previously described [51,96].

### **2.2.17 Statistical analysis**

All statistical analyses were conducted using MATLAB. We assessed the normality of each data set with the Shapiro-Wilk test. For normally distributed data, we used parametric tests, including paired t-test. For non-normally distributed data, the Mann-Whitney U test was applied for independent groups and the Wilcoxon signed-rank test for paired samples. Chi-squared tests were used to analyze binary data. Two-way ANOVA was employed to examine the effects of two independent variables and their interaction. Nested data were analysed with (generalized) linear mixed-effects models with mouse as random effect. A significant level of  $p < 0.05$  was considered for all tests.

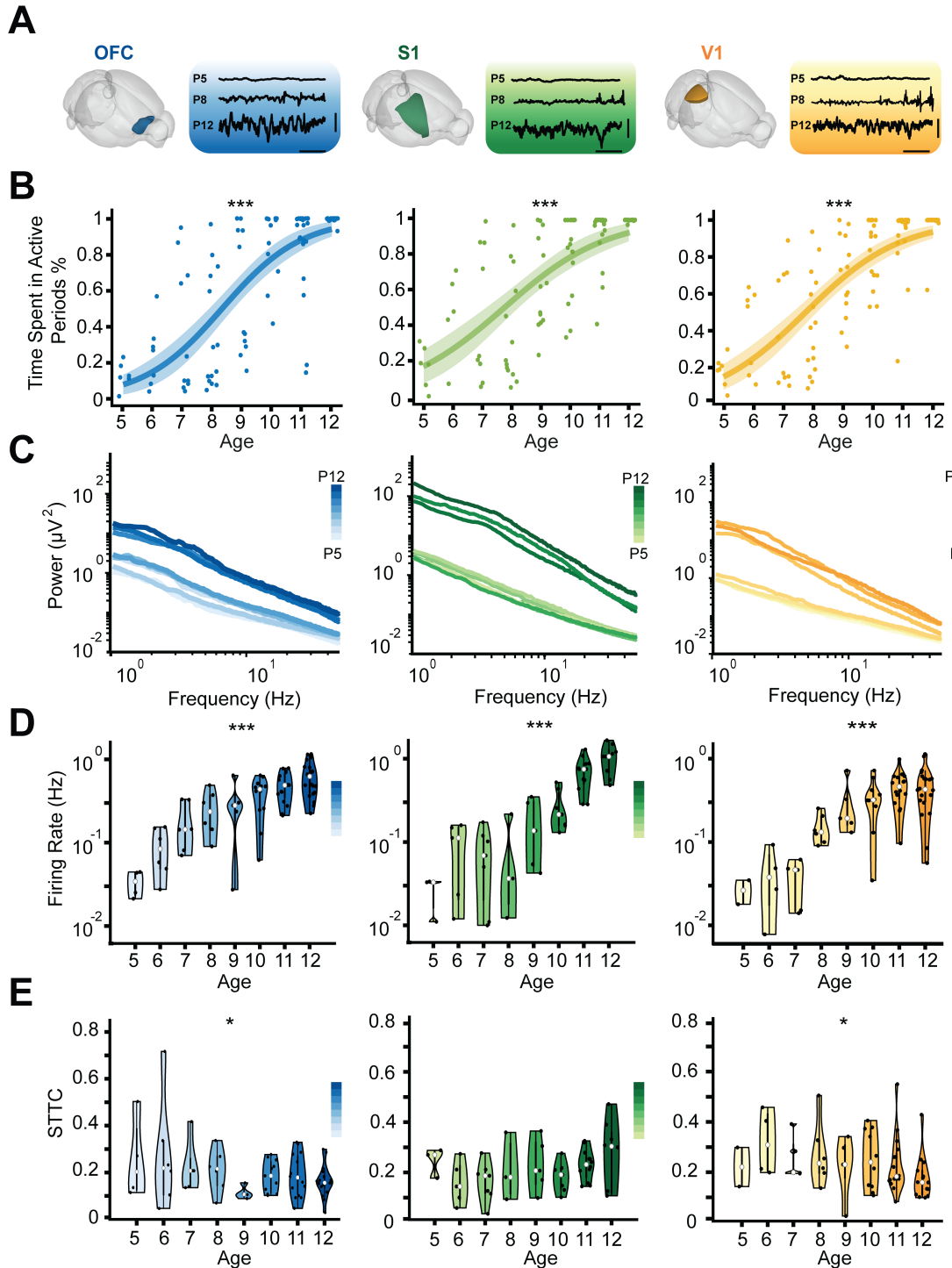
### 3 Results

#### 3.1 OFC, V1, and S1 develop similarly during the first weeks of neonatal development

To compare developmental activity dynamics across cortical hierarchical levels, we performed simultaneous multi-site extracellular *in vivo* recordings of local field potentials (LFP) and single-unit activity (SUA) from OFC, V1, and S1 in mice aged P5-P12 (Figure 4A). Across this developmental period, the LFP activity evolves from an activity pattern that primarily consists of silent periods with transient bouts of neuronal activity (active periods) to a continuous activity profile.

To quantify this transition in all areas, we assessed multiple measures of network activity. We measured the proportion of time spent in active periods, defined as epochs in which LFP activity exceeds a certain activity threshold, and we found that it increased monotonically with age across all three areas (OFC in blue:  $\beta = 0.13$ ,  $p < 0.001$ ; S1 in green:  $\beta = 0.116$ ,  $p < 0.001$ ; V1 in yellow:  $\beta = 0.117$ ,  $p < 0.001$ ; Figure 4B). In parallel with this shift in activity levels, we also found that broadband LFP power increases in all three areas (Figure 4C) and that SUA firing rate increase similarly over age in all three areas (OFC in blue:  $\beta = 0.085$ ,  $p < 0.0001$ ; S1 in green:  $\beta = 0.131$ ,  $p < 0.0001$ ; V1 in yellow:  $\beta = 0.063$ ,  $p < 0.0001$ ; Figure 4D).

Furthermore, to assess developmental changes in pairwise spike-time coordination, we computed the local spike-time tiling coefficient (STTC) between recorded single units in each area, a measure of temporal correlation that is independent of firing rate (Figure 4E; [95]). Here we found a significant decrease in STTC in both V1 and OFC over age (OFC in blue:  $\beta = -0.013$ ,  $p < 0.05$ ; V1 in yellow:  $\beta = -0.014$ ,  $p < 0.05$ ; Figure 4E), indicating that activity gradually decorrelates in the first neonatal weeks. Compared to V1 and OFC, S1 exhibited a flatter developmental trajectory of STTC over the same developmental time period (S1 in green:  $\beta = 0.010$ ,  $p = 0.075$ ; Figure 4E).



**Figure 4. Comparative developmental profiles of OFC, V1, and S1 during early postnatal development.** (A) Schematic depicting the *in vivo* recording paradigm with the neonatal OFC, S1, and V1 depicted in blue, green, and yellow, respectively, along with example traces of LFP (4-100 Hz) recorded from V1 (yellow), S1 (green), and OFC (blue) of P5, P8, and P12 mice. (B) Line plot displaying the percentage of time spent in active periods of P5-P12 mice ( $n = 106$ ) for OFC in blue (left), S1 in green (middle), and V1 in yellow (right). (C) Line plots displaying the log-normalized broadband power spectral density (PSD) in the 1-40 Hz frequency range of P5-P12 mice ( $n = 106$ ) for OFC in blue, S1 in green, and V1 in yellow. (D) Violin plots displaying the single unit activity (SUA) firing rate of P5-P12 mice ( $n = 68$ ) for OFC in blue, S1 in green, and V1 in yellow. (E) Same as (D) but for STTC. \*\*\* $p < 0.0001$ , \* $p < 0.05$ . Statistical tests: Generalized linear model (B), (D), and (E).

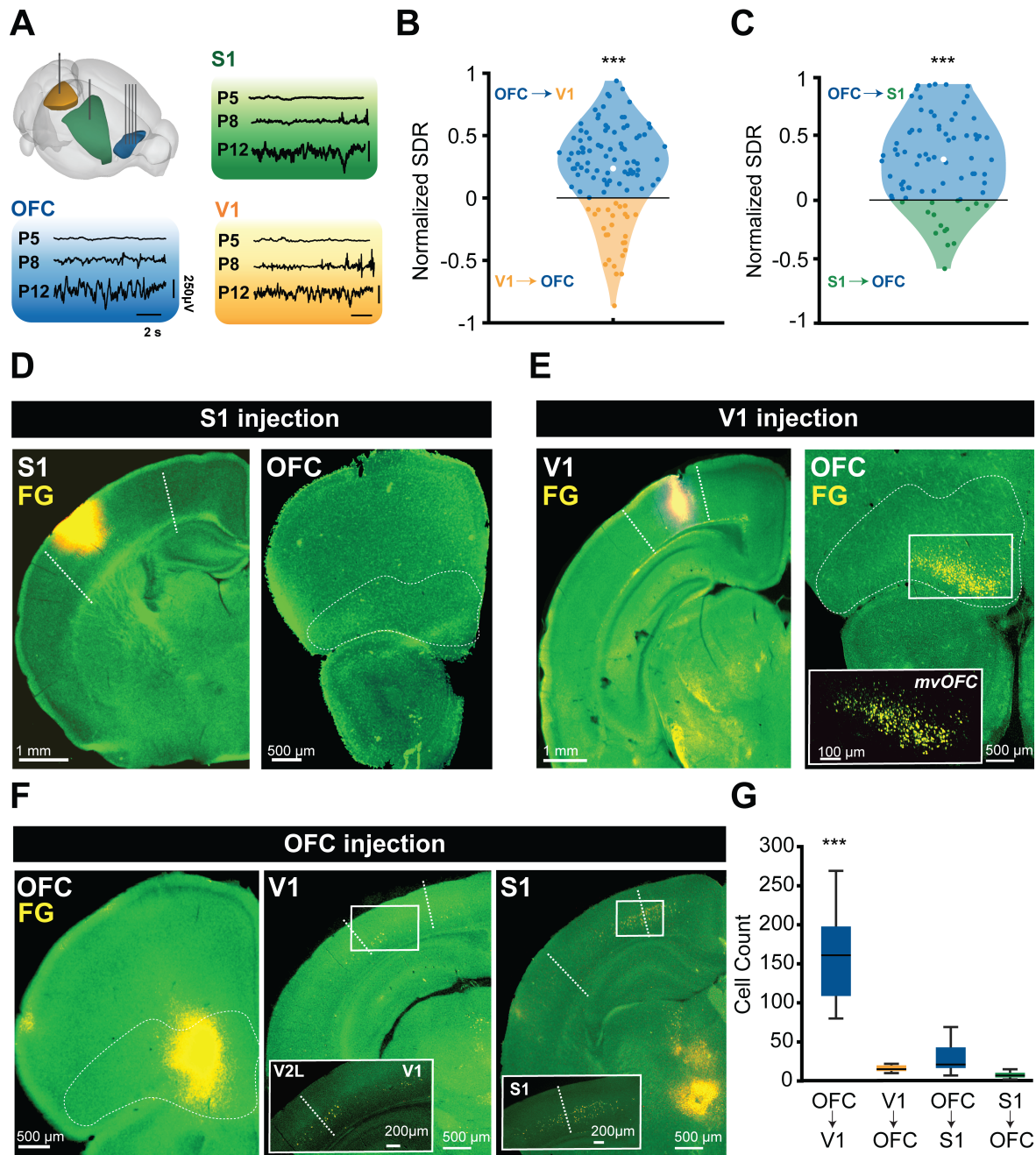


Together, these results show that OFC, V1, and S1 share a similar developmental activity profile over the first postnatal weeks, shifting from activity dominated by transient bouts separated by periods of network silence to an overall increase in broadband LFP power, SUA, and time spent in active periods. Importantly, these findings indicate that, at the level of overall activity metrics, OFC does not exhibit a delayed onset of network activation compared to primary sensory cortices during early development but instead undergoes similar early transitions in activity dynamics.

### **3.2 Early emergence of top-down functional coupling and preferential monosynaptic projections from OFC to V1**

Having established that OFC, V1, and S1 exhibit comparable baseline developmental activity profiles during the first two postnatal weeks (Figure 4), we next asked whether these regions already show evidence of functional and anatomical coupling at this early developmental stage. We examined whether directed interactions between OFC and primary sensory cortices are already present.

Combining electrophysiological and anatomical approaches, we investigated whether top-down pathways from OFC to sensory cortices are active during early development (Figure 5A). First, we performed multisite *in vivo* extracellular recordings of local field potentials simultaneously from OFC, V1, and S1. Leveraging the Spectral Dependency Ratio (SDR) to measure the directionality of information flow, we found that in most P5-P12 mice, the dominant direction of information flow between OFC and V1 was biased in a top-down manner (OFC->V1;  $n = 79/106$  mice;  $p < 0.0001$ ; Figure 5B). A similar directional bias was also observed between OFC and S1 (OFC->S1;  $62/77$  mice;  $p < 0.0001$ ; Figure 5C), indicating that top-down functional coupling from OFC to sensory cortices is already present during the first neonatal weeks.



**Figure 5. Top-down functional coupling from OFC to sensory cortices and preferential monosynaptic projections to V1.** (A) Top, schematic of the *in vivo* recording paradigm with the neonatal V1, S1 and OFC depicted in yellow, green and blue, respectively (top). Bottom, example traces of LFP (4-100 Hz) recorded from V1 (yellow), S1 (green), and OFC (blue) of P5, P8, and P12 mice. (B) Violin plot displaying the normalized SDR ( $n=106$  mice) between OFC and V1. (C) Same as (B) but for OFC and S1 ( $n = 77$ ). (D) FG injection site in S1 (left) and retrogradely labelled neurons in the OFC of a P12 mouse (right). (E) Same as (D) for FG injections in V1 and retrogradely labelled neurons in OFC. (F) Same as (D-E) for FG injections in the OFC and retrogradely labelled neurons in V1 and S1. (G) Box plot displaying the number of FG-labelled cells for the three injection sites ( $n=6$  mice) corresponding to OFC neurons projecting to V1 (OFC→V1), OFC neurons projecting to S1 (OFC→S1), and V1/S1 neurons projecting to OFC (V1/S1→OFC). \*\*\* $p < 0.0001$ . Statistical tests: binomial test in (B) and (C), Welch's t-test in (G).

We next examined whether this directional coupling is reflected in the underlying anatomical connectivity between OFC, V1, and S1. For this, we injected the retrograde tracer FG into V1, S1, or OFC and quantified labeled neurons in connected regions. FG injections into V1 resulted in robust labeling of neurons in the OFC, indicating a strong projection from OFC to V1 (Figure 5E). In contrast, FG injections into S1 yielded little to no labeling in OFC (Figure 5D), suggesting that projections from OFC to S1 are comparatively sparse. Finally, FG injections into OFC did not result in consistent labeling in either V1 or S1, indicating a weak monosynaptic bottom-up connection (Figure 5F). Quantification of labeled neurons confirmed that projections from OFC to V1 are significantly stronger than those involving S1, or monosynaptic bottom-up projections ( $t(8.07) = 6.83$ ,  $p < 0.0001$ ; Figure 5G).

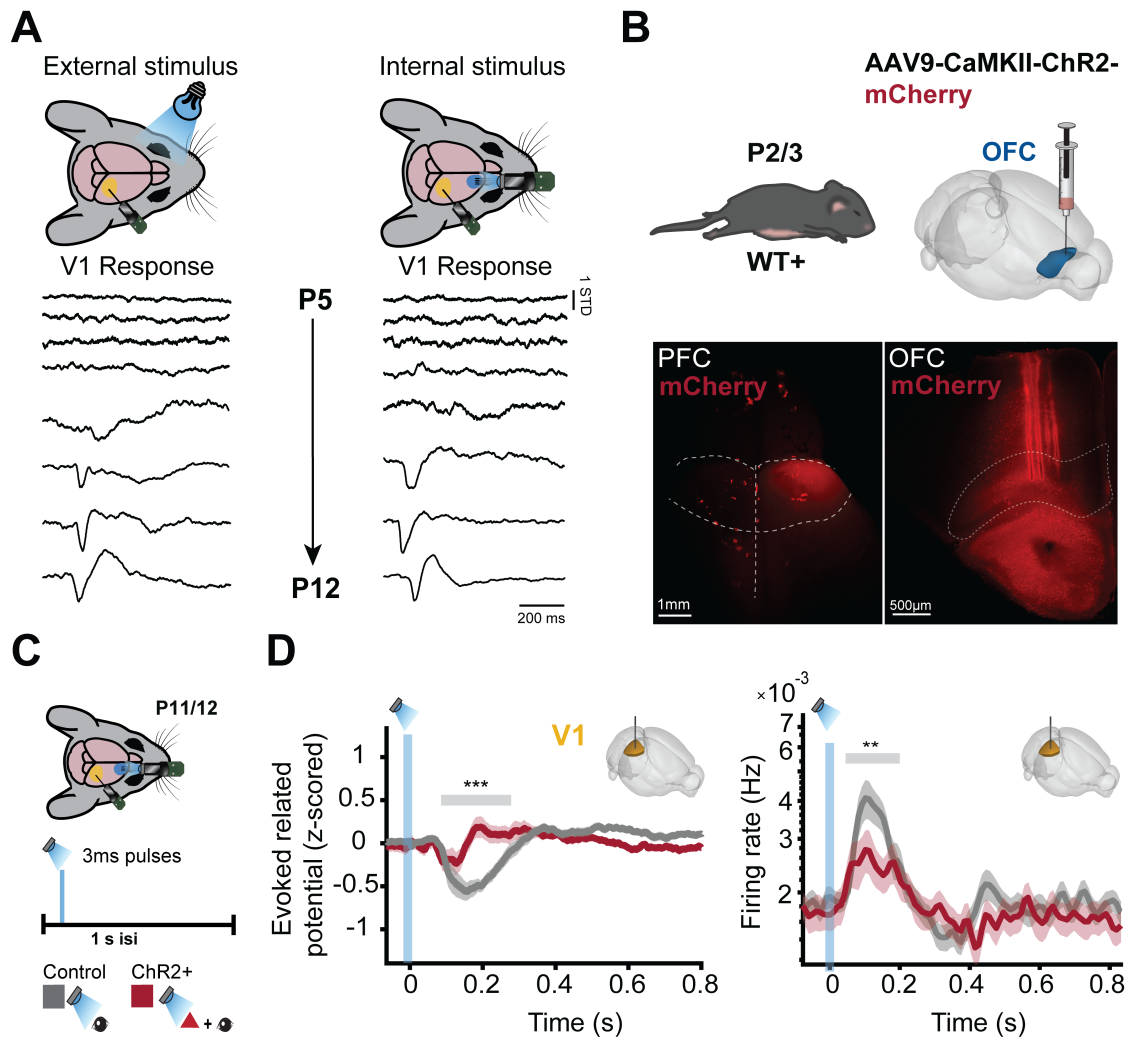
Taken together, these results indicate that while top-down functional interactions from OFC to sensory cortices are broadly present during early development, anatomical monosynaptic projections are selectively enriched toward V1.

### **3.3 Early OFC-V1 projections exert inhibitory control over visual response**

Next, we aimed to investigate the functional influence of OFC projections on V1-activity at this early neonatal stage. In adult mice, we know that OFC projections inhibit V1 activity by targeting SST – positive interneurons[78]. With this in mind, we know that at early developmental stage, firing rates are markedly low, making it challenging to detect potential inhibitory connections through spike train analysis. To overcome this, we increased V1 activity via light stimulation of the retina. Retinal ganglion cells are already light sensitive by P9–P10 [5], even when the eyelids are closed. Accordingly, an "external" visual stimulus, delivered via brief 3 ms-long pulses of blue light through closed eyelids led to prominent event-related potentials (ERPs) in P10-12 mice, yet not at earlier stages of postnatal development. Notably, the evoked ERPs are similar in their amplitude, duration and developmental dynamics to those evoked by an "internal" visual stimulus (i.e, blue light stimulation with the same properties delivered via an optic-fiber implanted in the frontal cortex) (Figure 6A). Similar optical stimulation-evoked activity has been recently reported in adult mice and has been attributed to propagation of light to the back of the eye, where it activates endogenous opsins within

the retina [97]. Thus, V1 activation can be reliably achieved by “internal” blue light stimulation in neonatal mice lacking transfected opsins.

Next, we used previously developed protocols [64,98] and injected an AAV carrying an excitatory opsin under the CaMKII promoter into the OFC of P2/3 mice, achieving robust expression by P9–P10 (Figure 6B). Brief blue light stimuli, with the same properties as those used for the “internal” V1 stimulation, were delivered to the OFC in mice with virus injection, and controls without virus in the OFC (Figure 6C). Transfected mice yielded a robust optogenetic activation of the OFC, with optogenetic stimulation resulting in large negative ERPs that were accompanied by an increase in spiking activity (Supp. Figure 1A). When compared to the “internal” activation of V1 (i.e, light only, no opsin in OFC), the OFC stimulation altered the V1 light-evoked ERP in two distinct ways. First, the negative deflection associated with the visual stimulus (~100-200 ms post-stimulus) was shortened (Figure 6D, left,  $U=1$ ,  $p<0.0001$ ). Second, a positive deflection (~200 ms post-stimulus) emerged, which was absent in non-transfected mice. Thus, OFC stimulation resulted in a shortened sensory-evoked excitatory ERP in V1, which was followed by an inhibitory ERP. Analysis of the evoked firing rates yielded compatible results. The visual stimulation alone elicited a robust increase in V1 spiking activity, with a peak at ~100 ms after light onset. When paired with the optogenetic OFC stimulation, the stimulus-evoked spiking peak was significantly attenuated (Figure 6D, right,  $U=141$ ,  $p=0.007$ ). These results indicate that, even during early development, OFC projections to V1 are functional and inhibit sensory responses.



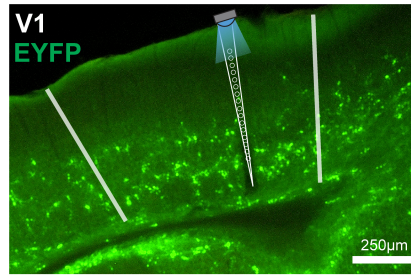
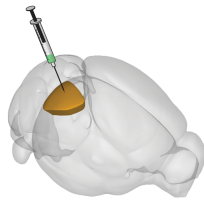
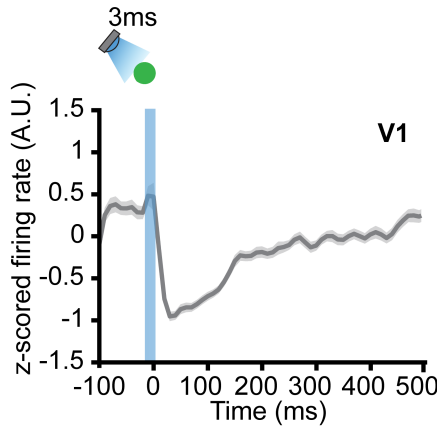
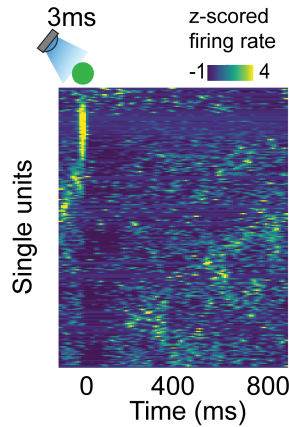
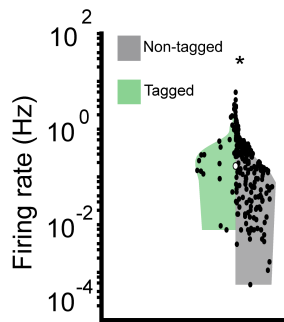
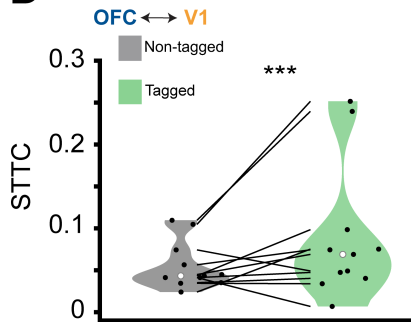
**Figure 6. Optogenetic activation of OFC inhibits V1 activity at neonatal age.** (A) Top, schematic illustrating the external and “internal” visual stimulation paradigms. Bottom, corresponding ERPs recorded in P5-12 mice. (B) Top, schematic of OFC-transfection using AAV9-CaMKII-ChR2-mCherry in a neonatal mouse. Bottom, brain-wide mCherry expression with delineation of frontal lobe on both hemispheres (dotted lines) (left) and mCherry expression in the OFC (marked by dotted lines) together with the fluorescently-marked optrode trace (bottom right). (C) Schematic of the optogenetic stimulation paradigm. (D) Line plots displaying z-scored ERPs (left) and firing rates (right) elicited in the V1 of the controls (no opsin, gray, n=10) and OFC-transfected mice (red, n=7) in response to blue light stimulation. Shadow areas correspond to significant differences in the ERP (left) and evoked firing rates (right). \*\*\* $p < 0.0001$ , \*\* $p < 0.01$ . Statistical test: Mann-Whitney U test.

### **3.4 Early OFC-to-V1 projections preferentially engage SST+ interneurons**

To uncover the cellular substrate of the observed inhibitory OFC-V1 interaction, we tested the hypothesis that, as in adult mice, the neonatal top-down inhibition is mediated by V1 SST interneurons. For this, we investigated SST-cre mice that were injected with a cre-dependent AAV-virus carrying EYFP and an excitatory opsin in V1 (Figure 7A). Consistent with prior reports on the spatial distribution of SST interneurons in the developing V1 [50,99], we observed that SST expression was mainly confined to infragranular layers (Figure 7A, right). The targeted SST neurons in V1 exhibited robust spiking responses to brief light pulses, allowing reliable phototagging of this population (Figure 7B; Supp. Figure 2A & B). Of note, optogenetic stimulation of SST interneurons suppressed spiking activity in V1 for approximately 200 ms, consistent with an inhibitory effect of interneurons already at neonatal ages [50,51,67,100–102] (Figure 7B). When compared to other V1 neurons, the phototagged SST interneurons displayed elevated baseline firing rates (Figure 7C,  $\beta = 0.307$ ,  $p = 0.049$ ). To assess the task-free communication between V1 and OFC, we calculated the spike-time tiling coefficients (STTC) between spike trains of the two brain areas, a measure of pairwise neuronal correlation. We found that phototagged SST interneurons in V1 had significantly higher STTC values with OFC spike trains when compared to non-phototagged V1 units (Figure 7D,  $\beta = 0.0279$ ,  $p < 0.0001$ ). These findings suggest that the top-down OFC projections engage and potentially target SST interneurons in V1 during early development.

**A**

AAV9-Ef1a-DIO  
hChR2(E123T/T159C)-  
EYFP

**B****C****D**

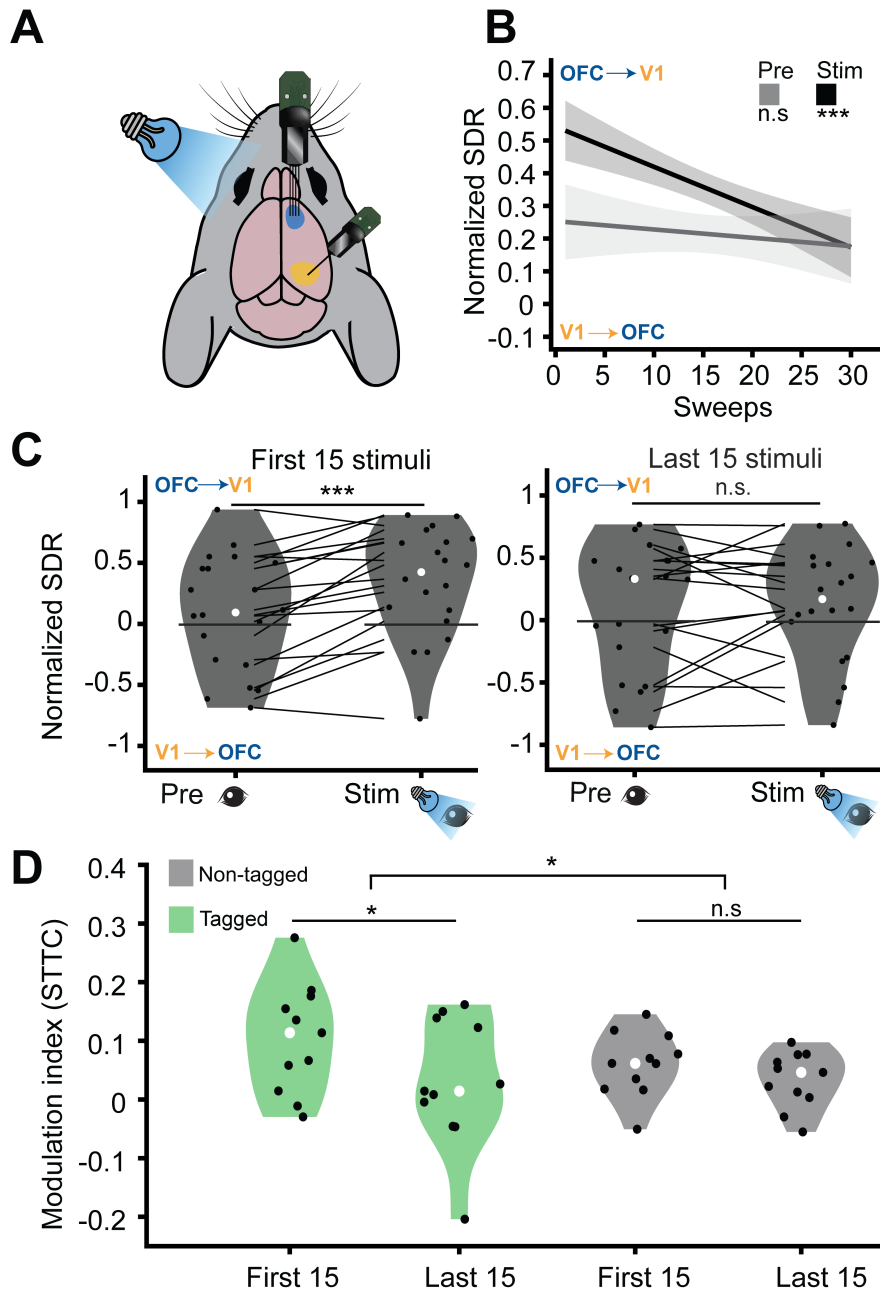
**Figure 7. Role of SST interneurons for the top-down OFC-V1 communication.** (A) Left, schematic of the SST-confined transfection with AAV9-Ef1a-DIO-hChR2(E123T/T159C)-EYFP in SST Cre<sup>+</sup> mice. Right, coronal sections from a P12 Cre<sup>+</sup> mouse displaying EYFP expression largely confined to SST interneurons in infragranular layers. (B) Left, color-coded raster plot displaying V1 single unit activity (SUA) in response to optogenetic activation of SST interneurons in V1. Note the pronounced silencing of firing after stimulus in the majority of neurons. Right, z-scored V1 firing rate in response to optogenetic activation of SST interneurons in the OFC of P9-12 mice (n=11). (C) Violin plot displaying the firing rate of phototagged (green, n=22 units) and non-phototagged (gray, n=276 units) V1 units. (D) Violin plot displaying the average STTC between OFC and phototagged (green, n=593 unit pairs) and non-phototagged (gray, n=5777 unit pairs) V1 units. Asterisk in (C) and (D) indicates significant differences between phototagged and non-phototagged units. \*\*\* $p < 0.0001$ , \* $p < 0.05$ . Statistical test: linear mixed effects model in (C-D).

### 3.5 OFC-V1 projections are specifically activated by behaviorally salient stimuli

Next, we asked whether we could engage the early top-down communication from the OFC to V1 SST interneurons by presenting a behaviorally salient stimulus (Figure 8A). Contralateral eye stimulation led to initially increased OFC→V1 SDR values, indicative of enhanced top-down communication, that faded over repeated sensory stimulations. While SDR remained largely stable in the pre-stimulus period, during the sensory stimulation it diminished with repeated exposure (Figure 8B, pre-stimulus period  $\beta = -0.002$ ,  $p = 0.45$ ; during stimulus period  $\beta = -0.012$ ,  $p < 0.001$ ). Accordingly, the SDR increase was significant for the first 15 sweeps of visual stimulation, when the stimulus novelty was at its peak, but not during the last 15, when stimulus novelty is diminishing (Figure 8C, left:  $t(19) = -4.495$ ,  $p < 0.0001$ ; right:  $U = 400$ ,  $p = 0.797$ ). This suggests that the increased communication from the OFC to V1 reflects the novelty and the behavioral relevance of the stimulus, and not simply sensory information.

To corroborate this result, we analyzed the V1 and OFC single unit response to sensory stimulation. V1 units, unlike those in the OFC, responded to the visual stimulus with an increase in firing rate (Supp. Figure 3A; Figure 8A). More importantly, we observed that this led to an increase in STTC values between OFC and V1 units, as measured by the STTC modulation index (MI, defined as  $[STTC_{stim} - STTC_{pre}] / [STTC_{stim} + STTC_{pre}]$ ) (Supp. Figure 3B). This increase was stronger for phototagged than non-phototagged units ( $\beta = 0.0349$ ,  $p = 0.0034$ ) (Figure 8D). Moreover, while the overall STTC MI was not higher in the first than the last sweeps of the visual stimulation ( $\beta = -0.0040$ ,  $p = 0.4692$ ), this was the case for the phototagged units (interaction effect:  $\beta = -0.0366$ ,  $p = 0.0292$ ; Post-hoc comparisons: phototagged:  $p = 0.0106$ , non-phototagged:  $p = 0.4692$ ) (Figure 8D). This increase paralleled the enhanced OFC-to-V1 SDR, suggesting that SST interneurons might mediate this increased communication and confirming the cell-type specificity of the interaction between the two brain areas at neonatal age.





**Figure 8. Top-down OFC-V1 communication is modulated by contralateral visual stimuli that engage V1 SST+ interneurons.** (A) Schematic of the experimental paradigm. (B) Normalized SDR before (light gray) and during (black) stimulus presentation as a function of the number of stimulus presentations (n=20 mice). (C) Violin plots displaying the normalized SDR between OFC and V1 before and during the stimulus presentation for the first (left) and last (right) 15 visual stimulations (n=20 mice). (D) Violin plots displaying the STTC modulation index for the first 15 and last 15 stimulus presentations between OFC and phototagged V1 units (green, n=11 mice) and OFC and non-phototagged V1 units (gray, n=11 mice). \*\*\*p<0.001, \*p<0.05. Statistical tests: Generalized linear model (B), paired t-test, left, and Mann-Whitney U test, right, in (C), linear mixed effects model in (D).

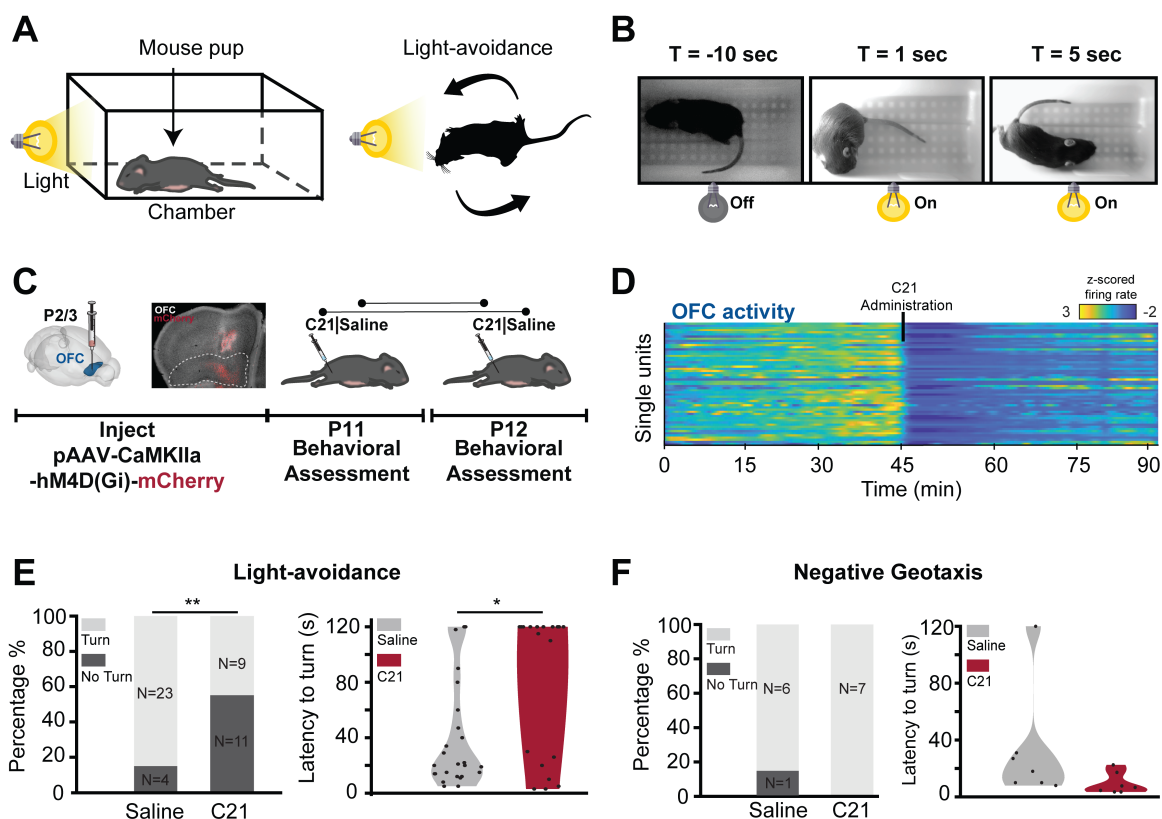
These findings reveal that, already during early postnatal development, the OFC exerts top-down control on V1 via monosynaptic projections targeting SST interneurons and shapes sensory processing before the onset of visual experience. This projection is not broadly activated by visual stimuli, but rather only by stimuli that carry salient behavioral relevance.

### **3.6 OFC inhibition specifically impairs early light aversion behavior**

Next, we wanted to investigate whether the OFC is involved in regulating light-avoidance behavior, a visually dependent innate behavior observed in mice at neonatal age in which they turn away from bright light sources (Figure 9A-B). To investigate this, we chemogenetically silenced the OFC pyramidal neurons and monitored the behavioral consequences. For this, we injected an inhibitory DREADD hM4D(Gi) expressed under the CaMKII promoter into the OFC of P2-3 mice (Figure 9C, left). To investigate the effects of C21 administration on OFC neural activity, we recorded the SUA of OFC after C21 injection and observed a strong reduction in spiking (Figure 9D), confirming the inhibitory effects of the DREADDs in combination with the C21 agonist. To assess the impact of the manipulation on light avoidance, we used a cross-over design, in which this behavior was probed twice in each mouse, at P11 and P12. Half of the mice received C21 before testing on the first day, and saline before the second day, while the other half received the protocol in reverse order (Figure 9C, right). Similar to previous studies [22], we defined light avoidance as the mouse turning 180° away from a light source within 120 seconds (Figure 9B). Approximately 85% of control mice (n=23/27 mice) exhibited this behavior, while only 45% (n=9/20 mice) of C21-treated mice did the same (Figure 9E, left;  $\chi^2(1) = 8.54$ ,  $p = 0.003$ ). Accordingly, the latency to turn was significantly longer in C21-treated mice (Figure 9E, right; Group:  $F(1, 36) = 8.53$ ,  $p = 0.006$ ). The behavioral effects were age-independent (Supp. Figure 4A, Age:  $F(1, 36) = 2.89$ ,  $p = 0.098$ ; Age×Group:  $F(1, 36) = 0.28$ ,  $p = 0.603$ ).

To rule out potential nonspecific motor effects after C21 administration and OFC inhibition, which might hamper mice to turn away from the bright light, we monitored the negative geotaxis reflex – a behavior in which mice reorient against gravity when placed on a 45° inclined surface (Figure 9F, left). Control and C21-treated mice performed the task with comparable success rates (6/7 control; 7/7 C21, Figure 9F;  $\chi^2(1) = 1.076$ ,  $p = 0.299$ ) and latencies (Figure 9F, right,  $p = 0.125$ ), indicating that both

groups of mice have comparable motor abilities. These results indicate that chemogenetic silencing of OFC specifically impairs light-avoidance behavior without broadly disrupting motor behaviors. This challenges the prevailing view that HOAs, such as the OFC, develop too slowly to be behaviorally relevant during the first postnatal weeks. The present data indicate that the OFC is critical for executing an early and evolutionarily important behavior such as neonatal light-avoidance behavior.



**Figure 9. OFC modulates intrinsic light-avoidance behavior in neonatal pups.** (A) Schematic of the testing arena for the light-avoidance in neonatal mice. (B) Illustration of the typical behavior of light-avoidance in neonatal mice (described in detail in *Methods*). (C) Schematic of the experimental approach displaying the site of injection of the AAV9-CaMKII-hM4D(Gi)-mCherry viral compound in P2-3 mice, followed by behavioral assessment at P11-12. (D) Color-coded raster plot displaying the recorded single unit activity in OFC before and after C21 administration in P11 in a virus-injected mouse. (E) Left, bar plot showing the number and percentage of mice that performed the light-avoidance behavior for the saline- (n=27 mice) and the C21-treated (n=20 mice) groups. Right, violin plots showing the latency to turn for saline- (n=27) and C21-treated (n=20 mice) mice. (F) Same as (E) for the negative geotaxis assay (n=7 mice for saline- and n=7 for C21-treated groups). Asterisks in (E) and (F) indicate a significant effect of C21 administration. \*\* $p < 0.01$ , \* $p < 0.05$ . Statistical tests: Chi-squared test in (E, left and F, left), two-way ANOVA in (E, right), Wilcoxon test in (F, right).

## 4 Discussion

The goal of the work presented in this thesis was to investigate how interactions between HOAs and LOAs emerge during early neonatal development, and whether such interactions are already present and contribute to regulating sensory processing and behavior at neonatal stages. While traditional models assume that early cortical development is largely dominated by a bottom-up sensory drive, the extent to which top-down influences are present and even functional during neonatal stages remains poorly understood. Here, we present evidence that top-down signals from OFC are already anatomically established, functionally active, and behaviorally relevant during early neonatal development, suggesting that HOAs involvement in sensory processing and behavioral control may emerge earlier than traditionally assumed.

Our initial investigations aimed to characterize the baseline developmental trajectories of neural activity across two primary sensory areas, V1 and S1, and a HOA, the OFC. Across several electrophysiological metrics, including time spent in active periods, PSD, SUA, and STTC, all three areas exhibited broadly similar developmental trends. While this does not contradict the well-established idea that HOAs like the OFC have a protracted developmental maturation profile, they indicate that, at the basic level of ongoing network activity, all three areas already follow similar developmental dynamics. This suggests that HOAs may be more functionally engaged at an earlier developmental timepoint than traditionally assumed.

Beyond these shared developmental dynamics, we then identified monosynaptic projections linking OFC to V1. Monosynaptic projections from OFC to V1 were already present at neonatal stages and were more pronounced than projections from OFC to S1, indicating a target specificity in early top-down connectivity. This selective anatomical finding suggests that the cortical output of HOAs like the OFC may preferentially engage specific modalities in a top-down dominant manner. In contrast, bottom-up monosynaptic projections from V1 and S1 targeting the OFC were sparse in comparison, suggesting that early cortico-cortical communication is not equally bidirectional, but instead biased from HOAs towards LOAs, in a top-down manner. Interestingly, this anatomical asymmetry was paralleled by functional measures of interconnective communication between brain areas. Analysis of SDR, a PSD-derived measure, revealed a top-down bias in the direction of

communication between OFC, V1 and S1, indicating that HOAs like the OFC already exert a measurable influence on primary sensory regions. However, when considered together with the anatomical data, these findings highlight a distinction between neural activity-level top-down interactions and their underlying anatomical connectivity. While top-down dynamics are detectable across multiple sensory areas, the underlying structural connectivity appears to be more strongly established between OFC and V1. Taken together, these results indicate that during early neonatal development, HOAs and LOAs are organized in an asymmetric, selective, and top-down-dominant manner, rather than reciprocal or uniformly distributed.

Building on the anatomical findings, we showed that top-down communication from OFC to V1 is functionally active during early neonatal development. We combined sensory stimulation with optogenetic activation of OFC excitatory neurons. Using a light-stimulation paradigm that allowed simultaneous activation of a visual response and transfected OFC neurons, we found that OFC activation markedly altered the visual response in V1. Specifically, the sensory-evoked negative deflection of the ERP response was shortened, indicating a shift toward increased inhibitory network activity. Consistent with this, stimulus-evoked SUA was significantly reduced when OFC was activated.

These findings demonstrate that, even at early developmental stages, OFC projections exert a functional influence on sensory processing in V1 by suppressing visually evoked activity. Thus, early top-down signals are not merely present anatomically but are capable of dynamically shaping cortical responses to external stimuli.

To identify the cellular substrate underlying OFC-V1 interactions, we next examined the role of SST-expressing interneurons in V1. SST interneurons were already functionally active during neonatal development and exerted a strong inhibitory influence on local V1 network activity. Notably, V1 SST interneurons showed a stronger spike-time coupling to OFC units than other V1 units, suggesting they are preferentially engaged by top-down input. Together, this indicates that SST interneurons represent a likely cellular target of OFC projections in the developing V1, pointing to a potential substrate for top-down interactions at early developmental stages.

Given that SST interneurons are a likely target in V1 for OFC input, we next examined whether early OFC-V1 communication is engaged during a visual

stimulation paradigm. Visual stimulation led to a transient increase in top-down communication between OFC and V1, as reflected by the elevated SDR values during the stimulation period. This effect diminished with repeated stimulation, while pre-stimulus activity remained stable, indicating that OFC-driven signaling is dynamically modulated by stimulus exposure rather than reflecting a constant level of coupling between OFC and V1.

At the level of single units, the visual stimulation paradigm increased spike-time coupling between OFC and V1 neurons. Notably, this increase was more pronounced between OFC and V1 SST interneurons, and exhibited a similar dependence on repeated stimulation, mirroring the dynamic patterns observed at the population level. Together, these findings suggest that early OFC-V1 communication is transiently recruited during a visual stimulation paradigm, and preferentially engage V1 SST interneurons, further supporting their role as a key substrate for top-down interactions in developing V1.

Finally, we examined whether OFC activity contributes to early light avoidance behavior in neonatal mice. Chemogenetic silencing of OFC neurons significantly reduced the innate light-avoidance behavior in neonatal mice. Importantly, this effect was not accompanied by deficits in general motor abilities, indicating that the observed reduction in light-avoidance behavior reflects a specific disruption of the behavior rather than non-specific motor deficits following the chemogenetic silencing.

These findings demonstrate that OFC activity plays a role in the regulation of the normal expression of early light avoidance behavior in neonatal pups. Together with our circuit-level results, this suggests that top-down signals from OFC are not only present during early development but also contribute directly to the expression of behaviorally relevant innate responses to visual stimuli.

## **4.1 Revisiting developmental cortical frameworks**

During early postnatal life, animals must respond appropriately to environmental challenges despite limited sensory and motor capabilities. Innate behaviors are thought to provide a necessary set of preconfigured behavioral responses that complement these developmental constraints, allowing effective responses to salient stimuli without prior experience or learning. Traditionally, neonatal innate behaviors are assumed to rely primarily on subcortical structures, while HOAs are thought to remain

largely dormant and functionally inactive during early development. This view is closely linked to the idea that HOAs are immature at neonatal stages and only become actively involved in behavioral regulation and cognitive processing during later developmental stages. This perspective is grounded in the well-established observation that cortical areas do not mature uniformly. A hierarchical pattern of cortical development has been widely observed and described, following gradients that align with anatomical, functional and evolutionary organization of the brain, and characterized by a non-uniform postnatal expansion across cortical regions [45,103,104].

Although cortical contributions to innate behavior have been recognized, they are typically seen as modulatory influences acting on fundamentally subcortical circuits, rather than being the primary generators of behavior. For instance, studies in visual circuits have shown that cortical outputs can shape innate behavioral responses, for example affecting light-induced arrest via projections from infragranular layers of V1 to downstream subcortical structures such as the superior colliculus (SC), which plays a key role in executing the behavior [105–107]. In this framework, subcortical regions provide the core circuitry for rapid and evolutionarily conserved innate behavioral responses, while cortical areas modulate the output in a context-dependent manner.

While this framework is well supported, our findings suggest that it may be incomplete. Our findings from the *in vivo* baseline recordings reveal that HOAs like the OFC and LOAs like V1 and S1 exhibit broadly similar developmental trajectories during early neonatal development. While these results do not contradict the established view of delayed structural and functional maturation of HOAs relative to LOAs, they do indicate that, at the level of ongoing network activity, HOAs and LOAs follow comparable developmental dynamics.

Building on this refinement of the traditional framework, our findings provide further evidence that early cortical networks are not solely organized in a bottom-up manner, in which LOAs primarily dictate cortical communication, but already exhibit structured top-down interactions. Notably, monosynaptic projections from OFC to V1, which have been described in the adult mouse brain [78], were present at neonatal stages, indicating that this top-down pathway emerges early in development rather than being a late-forming feature of mature cortical circuitry. Consistent with this, we observed that the dominant direction of communication has a top-down bias from OFC

to both V1 and S1. However, the underlying anatomical connectivity was more strongly established between OFC and V1, highlighting that early OFC-driven top-down organization is selective rather than uniformly distributed across sensory areas. Although OFC projections are not restricted to V1 and have been reported to interact with other sensory areas like S1 [77], we focused here on the OFC-V1 pathway given its anatomical specificity and the ability to directly assess its functional involvement in visually evoked responses.

Having identified early OFC-V1 projections and a bias towards top-down communication, we next examined whether these projections are functional.

## **4.2 Circuit mechanisms underlying OFC-V1 projections**

Top-down projections from HOAs are known to innervate sensory cortices and modulate sensory cortical activity through local inhibitory circuits [71]. In particular, projections from OFC to V1 have been shown to suppress visually evoked responses via preferential recruitment of V1 SST interneurons [78]. While such circuit relationships have been described in the adult mouse brain, it remains unclear when they emerge during development and whether they are already present and functionally active at neonatal stages.

To determine whether OFC-V1 projections are functionally active at neonatal stages, we combined sensory stimulation with optogenetic activation of OFC excitatory neurons. Using a light-stimulation paradigm that allowed simultaneous activation of a retinal visual response and optogenetic activation of transfected OFC neurons, we found that OFC activation markedly altered visually evoked responses in V1. Specifically, the sensory-evoked negative deflection of the ERP was shortened, and stimulus-evoked spiking activity was significantly reduced.

These findings indicate that OFC projections exert a net inhibitory influence on V1 activity already during early neonatal development, just at the onset of retinal light sensitivity. This demonstrates that this top-down pathway is not only anatomically present but also functionally active at neonatal age.

To identify the cellular substrate underlying this inhibitory effect, we next examined the profile of SST-positive interneurons in V1. We observed that SST interneurons were already functionally active at neonatal stages, exerting a strong inhibitory influence on local network activity, as reflected by a global suppression of V1



single-unit activity following SST activation. Notably, SST interneurons showed a stronger spike-time coupling with OFC units compared to non-SST V1 neurons, indicating a preferential functional relationship with top-down input.

SST interneurons are well established as key mediators of feedback inhibition in cortical circuits, regulating the gain and functional processing of sensory responses [108]. Consistent with this, projections from OFC have been shown to recruit SST interneurons to suppress activity in a primary sensory area [109]. Taken together, our findings suggest that SST interneurons represent a likely target for afferent OFC projections to exert an inhibitory influence on V1 activity during early development.

In this context, the OFC-V1 pathway is not only functionally active at neonatal ages but appears to be organized in a defined inhibitory circuit. In the adult mouse brain, this circuit architecture has already been described [78]. Our results suggest that key elements of this circuit architecture are already present during early neonatal development, with SST interneurons being a likely cellular substrate for OFC-driven inhibition of V1 activity.

These findings therefore indicate that the mechanisms underlying top-down modulation of sensory processing are not a late-emerging feature of mature cortical networks but are present and functionally engaged during early neonatal development.

### **4.3 Dynamic recruitment of OFC-V1 interactions during sensory stimulation**

Having established that OFC-V1 projections are functionally active and engage SST interneurons, we next examined under which conditions we could engage this pathway in a behaviorally relevant manner. To this end, we assessed the impact of a salient behaviorally relevant visual stimulation paradigm on OFC-V1 communication. Sensory stimulation led to a transient increase in the top-down directional influence from OFC to V1, as reflected by the increase in SDR values during the stimulation periods. Notably, this effect was strongest during the initial stimulus presentations and diminished with repeated stimulus exposure, while pre-stimulus activity remained stable across the paradigm. These findings indicate that early top-down communication between OFC and V1 is not continuously active but can be dynamically engaged with a behaviorally relevant stimulus, with a pronounced response during the initial exposure.

To further resolve the cellular basis of this stimulus-dependent engagement, we examined the interaction between OFC and V1 at the SUA level. Sensory stimulation increased the STTC between OFC and V1 neurons, indicating enhanced correlated activity between the two areas during stimulus presentation. Importantly, this increase was more pronounced for phototagged V1 SST interneurons compared to non-SST V1 units, pointing to a selective engagement of the V1 SST subpopulation.

Moreover, the temporal profile of spike-time coupling mirrored the dynamics observed at the population level. Sensory stimulation increased the STTC between OFC units and both phototagged SST units and non-phototagged units in V1, indicating a general engagement between OFC and V1 units during stimulus presentation. However, phototagged V1 SST interneurons exhibited a significantly stronger increase in spike-time coupling to OFC units when comparing the first 15 to last 15 stimulus presentations, whereas no comparable modulation was observed for non-phototagged V1 units.

Together, these findings indicate that early OFC-V1 interactions can be selectively recruited during sensory stimulation and show a pronounced engagement during initial stimulus exposure. While the STTC between OFC and V1 units is broadly increased during stimulation, only the STTC between OFC units and V1 SST interneurons shows a clear difference between the first and last 15 stimulus presentations, an effect not observed for non-SST V1 units. Importantly, these dynamics are already present at neonatal stages, suggesting that the OFC-V1 pathway is engaged in a context-dependent manner and may provide a mechanism through which top-down signals selectively shape sensory processing under conditions of behavioral relevance.

#### **4.4 Functional relevance of OFC-V1 interactions for innate behavior**

To determine the functional relevance of OFC-V1 interactions for behavior, we examined the effect of OFC inhibition on innate light-avoidance behavior in neonatal mice. Chemogenetic silencing of OFC excitatory neurons significantly impaired light-avoidance behavior, reducing the proportion of animals exhibiting the response and increasing the latency to turn away from the light source. Importantly, this effect was specific to light-avoidance, as motor performance remained unaffected, indicating that OFC activity is required for the proper execution of this innate, survival-relevant

behavior, rather than for general motor function. These findings demonstrate that, even at early developmental stages, top-down signals from the OFC contribute directly to the control of behavior in response to sensory stimuli that do not depend on prior learning.

In the adult mouse brain, top-down projections from HOAs modulate sensory processing in a context-dependent manner, allowing behavioral demands and internal states to shape incoming sensory information [70–72]. Within this framework, the OFC is extensively connected with subcortical regions, acting as a central hub for learning and value-guided decision making [81]. Moreover, it exerts top-down control through its connections with sensory cortices, including V1, where feedback from OFC has been shown to modulate visual encoding in a behavior-dependent manner and suppress sensory responses under specific conditions [77,78,109–111]. Our findings extend this framework by demonstrating that such top-down influences are not only present but already functionally engaged at neonatal stages and directly contribute to the execution of an innate behavior.

These findings raise the question of how OFC activity at neonatal stages influences the neural circuits underlying innate light avoidance. Unlike learned behaviors, innate responses rely on preconfigured circuits that can be engaged without prior experience. In the visual system, V1 has been implicated in the regulation of visually driven innate behaviors through its projections to subcortical structures such as the superior colliculus (SC), which play a central role in mediating defensive and avoidance responses [71,107,112–114]. Considering this, OFC is unlikely to directly generate behavioral output but rather modulate sensory representations in V1 that are related to downstream motor circuits.

Our data suggests that this modulation is mediated through an inhibitory mechanism, whereby OFC activity suppresses visually evoked responses in V1 via the recruitment of local V1 SST interneurons. Such suppression may serve to regulate the strength and timing of cortical output to subcortical and corticotectal targets, thereby shaping the behavioral response to visual stimuli. In adult mice, feedback from OFC has been shown to reduce visual encoding in V1 in a behavior-dependent manner, supporting a role for OFC in modulating, rather than driving, sensory processing [111]. In this context, OFC-driven inhibition of V1 activity in response to salient stimuli could

act to gate or refine the transmission of visual signals to downstream structures, including SC, ultimately influencing the execution of innate visually driven behaviors.

Notably, the dynamic engagement of OFC-V1 interactions observed in our data, characterized by strong initial responses that diminish with repeated stimulation, further supports this interpretation. This temporal profile suggests that OFC feedback preferentially operates under conditions of high stimulus saliency, where rapid behavioral responses are most critical. Together, these findings point to a model in which OFC does not initiate the innate light avoidance behavior *per se* but instead modulates early sensory processing to ensure that behaviorally relevant stimuli are appropriately transformed into adaptive motor responses during early development. This positions the OFC as a functionally active HOA that regulates behavior already during early neonatal development, challenging the view that such top-down control emerges only at later stages.

## **4.5 Limitations and future directions**

While the present findings provide evidence for an early functional role of OFC-V1 interactions in shaping innate behavior, several considerations define the boundaries within which these results should be interpreted. First, our assessment of baseline developmental dynamics was based on standard measures of neural network activity, which alone do not fully capture the broader maturation of higher-order cortical circuits. Accordingly, our findings do not contradict the established view that HOAs have a protracted neonatal developmental profile compared to LOAs on most metrics but rather indicate that key aspects of HOAs functional integration may emerge earlier than previously appreciated.

In addition, the involvement of SST interneurons in mediating OFC-V1 interaction is inferred from *vivo* recordings and analysis of functional coupling and therefore remains correlational. Although the selective engagement of SST interneurons strongly supports their role as a candidate cellular substrate of top-down modulation, direct anatomical or causal evidence linking OFC projections to SST interneurons in V1 is lacking.

Furthermore, the causal contribution of the OFC-V1 pathway to behavior cannot be directly isolated based on the current data. Chemogenetic silencing of OFC neurons affects the overall output of OFC neurons, rather than selectively targeting projections

to V1 or specific downstream cell types. As a result, while our findings clearly demonstrate that OFC activity is required for the execution of innate light-avoidance, they do not allow for a definitive attribution of this behavioral effect to OFC-V1 projections or to the recruitment of SST interneurons in V1. In addition, the proposed link between OFC-driven modulation of V1 activity and downstream subcortical circuits, such as the SC, remains indirect, as no direct data of corticotectal or subcortical activity were recorded.

Addressing these limitations will require pathway-specific and cell-type-specific manipulations, for example, by directly targeting the V1 units that receive OFC input in combination with behavior. Such approaches would enable a more precise dissection of the circuit mechanisms through which top-down signals influence sensory processing and the execution of innate behavior during early development.

Taken together, the present findings demonstrate that top-down interactions between OFC and V1 are already functionally engaged during early neonatal development, rather than only emerging at later stages of maturation. By linking OFC activity to both the modulation of sensory processing and the execution of innate, survival-relevant behavior, this work provides new insights into how HOAs contribute to behavioral control at a time when sensory systems are still developing. These results challenge the view of the immature brain as predominantly driven by bottom-up processes and instead support a model in which early top-down signals help shape the transformation of sensory input into adaptive behavior.

Future studies will be required to determine how these pathways mature over time and whether similar mechanisms operate across other modalities and behavioral contexts. Understanding these processes will be essential for clarifying how early cortical dynamics influence later cognitive functions and behavioral flexibility.

## 5 Summary

Innate behaviors are essential for survival, yet the developmental mechanisms underlying their emergence remain poorly understood. In mammals, the orbitofrontal cortex (OFC), a higher-order brain area, is thought to mature slowly relative to lower-order brain areas, contributing minimally, if at all, to behavior during early neonatal development. Here, neural activity recordings in neonatal mice (P5-12) reveal that the OFC follows a similar developmental trajectory to the primary visual cortex (V1) and the primary somatosensory cortex (S1). Furthermore, we show that the OFC already during neonatal development preferentially projects monosynaptically to V1 and that these projections, when activated, reduce the visual light response in V1. We then demonstrate that the OFC-V1 pathway is engaged by a behaviorally relevant sensory stimulation paradigm, during which V1 somatostatin-positive (SST) interneurons are preferentially recruited by OFC. Finally, we observe that chemogenetic inhibition of OFC excitatory activity reduces light-avoidance behavior in neonatal mice, an innate survival-critical behavior, even before eye opening. Our findings suggest that traditional views of cortical development may underestimate the early behavioral relevance of the OFC, identifying it as an active source of top-down sensory control already during early neonatal development.

## 6 Zusammenfassung

Angeborene Verhaltensweisen sind essenziell für das Überleben, doch die Entwicklungsmechanismen, die ihrer Entstehung zugrunde liegen, sind nach wie vor weitgehend unbekannt. Bei Säugetieren gilt der Orbitofrontalkortex (OFC) als ein übergeordnetes Hirngebiet, das im Vergleich zu untergeordneten Arealen langsamer reift und während der frühen postnatalen Entwicklung kein oder nur einen geringen Beitrag zum Verhalten leistet. Neuronale Aktivitätsaufzeichnungen in neonatalen Mäusen (P5-12) zeigen jedoch, dass der OFC einen vergleichbaren Entwicklungsverlauf wie der primäre visuelle Kortex (V1) und der primäre somatosensorische Kortex (S1) durchläuft. Darüber hinaus belegen wir, dass der OFC, bereits während der Neugeborenenentwicklung, bevorzugt monosynaptisch auf V1 projiziert und dass die Aktivierung dieser Projektionen die visuelle Lichtantwort in V1 abschwächt. Weiterhin zeigen wir, dass der OFC-V1-Pfad durch ein verhaltensrelevantes sensorisches Stimulationsparadigma aktiviert wird, in dessen Rahmen V1 Somatostatin-positive (SST) Interneurone präferentiell durch den OFC rekrutiert werden. Schließlich beobachten wir, dass die chemogenetische Hemmung exzitatorischer OFC-Aktivität das Lichtmeideverhalten neonataler Mäuse, ein angeborenes, überlebenswichtiges Verhalten, selbst vor der Augenöffnung reduziert. Unsere Ergebnisse legen nahe, dass etablierte Konzepte der kortikalen Entwicklung die frühe verhaltensrelevante Rolle des OFC unterschätzen, und identifizieren ihn als eine aktive Quelle top-down sensorischer Kontrolle bereits während der frühen postnatalen Entwicklung.

## 7 Abbreviations

|                   |                                                            |
|-------------------|------------------------------------------------------------|
| AAV               | Adeno-associated virus                                     |
| ANOVA             | Analysis of variance                                       |
| CA1               | Cornu ammonis 1                                            |
| CaMKII            | Calcium calmodulin-dependent kinase 2                      |
| ChR2              | Channelrhodopsin-2                                         |
| ChR2(E123T)       | Channelrhodopsin-2 E123T T159C                             |
| ChR2(H134R)       | Channelrhodopsin-2 H134R                                   |
| DAPI              | 4, 6-diamidino-2-phenylindole                              |
| DREADDs           | Designer receptors exclusively activated by designer drugs |
| E                 | Embryonic day                                              |
| E/I               | Excitatory/inhibitory                                      |
| ERP               | Event-related potential                                    |
| EYFP              | Enhanced yellow fluorescent protein                        |
| FG                | Fluorogold                                                 |
| GABA <sub>A</sub> | Type A $\gamma$ -aminobutyric acid                         |
| GD                | Day of gestation                                           |
| HOA               | Higher-order area                                          |
| Hz                | Hertz                                                      |
| ipRGCs            | Intrinsically photosensitive retinal ganglion cells        |
| IRES-Cre          | Internal ribosome entry site – Cre recombinase             |
| kHz               | Kilohertz                                                  |
| L1-6              | Cortical layer 1-6                                         |
| LFP               | Local field potential                                      |
| LGN               | Lateral geniculate nucleus                                 |
| LOA               | Lower-order area                                           |
| mA                | Milliampere                                                |
| MI                | Modulation index                                           |



|          |                               |
|----------|-------------------------------|
| NaCl     | Sodium chloride               |
| NP       | Neural progenitor             |
| OFC      | Orbitofrontal cortex          |
| P        | Postnatal day                 |
| PFC      | Prefrontal cortex             |
| PSD      | Power spectral density        |
| PV       | Parvalbumin                   |
| RG cells | Radial glial cells            |
| RGC      | Retinal ganglion cells        |
| S1       | Primary somatosensory cortex  |
| SC       | Superior colliculus           |
| SDR      | Spectral dependency ratio     |
| SST      | Somatostatin                  |
| Stim     | Stimulus                      |
| STTC     | Spike-time tiling coefficient |
| SUA      | Single-unit activity          |
| UV       | Ultraviolet                   |
| V1       | Primary visual cortex         |
| VZ       | Ventricular zone              |

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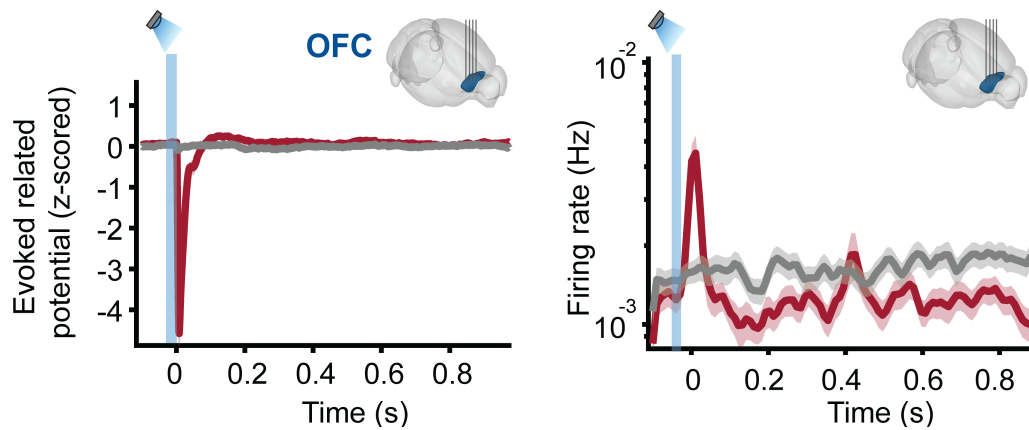
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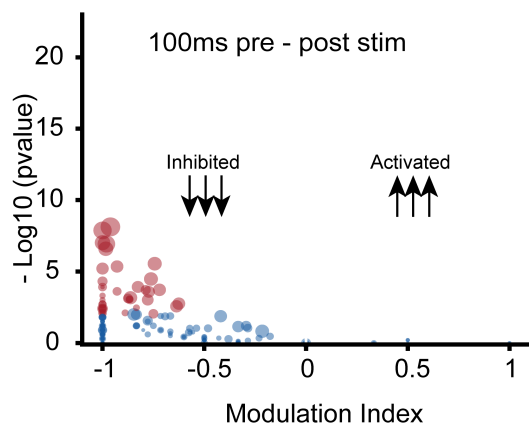
## Supplementary figures

**A**

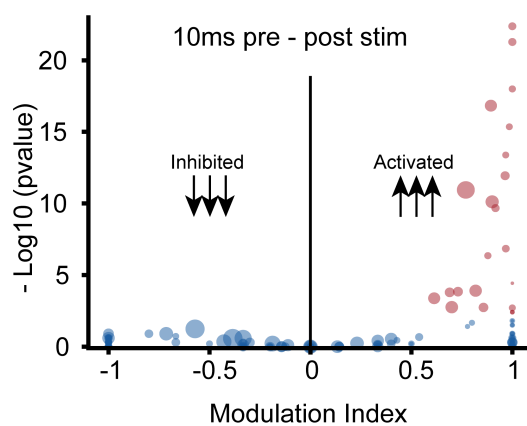


**Supplementary Figure 1. Consistent optogenetic activation of OFC in neonatal experiments. (A)** Left panel: Line plots displaying z-scored ERPs (left) and firing rates (right) elicited in the OFC of control mice (no opsin, gray n=10) and OFC-transfected mice (red, n=7) during simultaneous optogenetic and retinal stimulation. Right: Same as left, but for SUA responses.

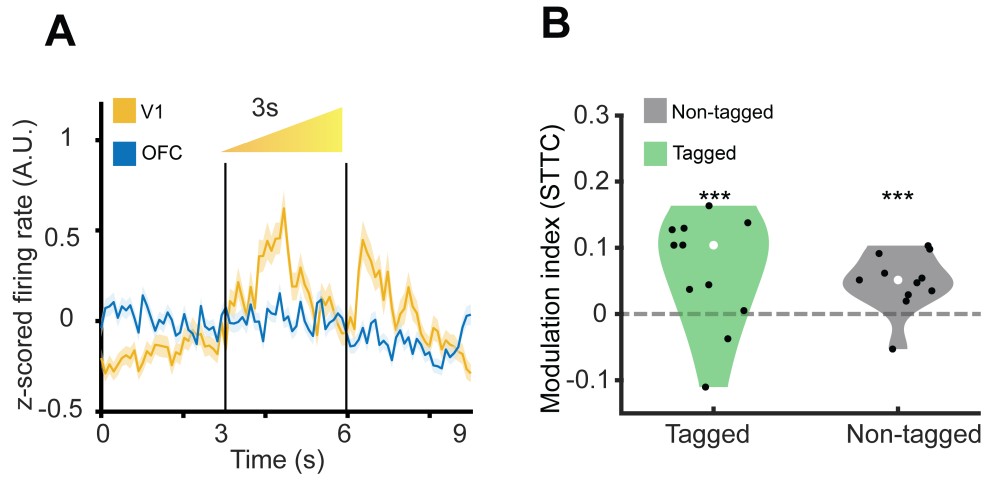
**A**



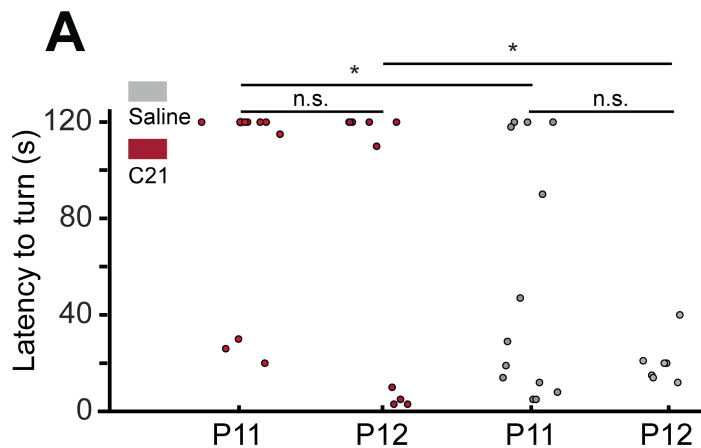
**B**



**Supplementary Figure 2. Initial optogenetic activation of SST interneurons causes widespread V1 inhibition. (A)** Modulation index (MI) comparing the 100 ms pre- to post- stimulation spiking response of V1 units following optogenetic activation of SST interneurons in virus-injected SST Cre+ mice. Positive MI values indicate increased firing activity, while negative values indicate decreased firing activity. Red dots denote significantly modulated units, and blue dots denote non-significantly modulated units. **(B)** Same as (A) but comparing the 10 ms pre- to post-pulse stimulation.



**Supplementary Figure 3. Visual stimulation reliably activates both phototagged SST interneurons and non-phototagged units in V1.** (A) Z-scored SUA of all V1 (yellow, n=298 units) and OFC (blue, n=320 units) units in response to 3-second retinal light stimulation. (B) Violin plots displaying the STTC modulation index for all stimulus presentations between OFC and phototagged V1 units (green, n=11 mice) and OFC and non-phototagged V1 units (gray, n=11 mice). \*\*\* $p < 0.001$ . Statistical tests: Linear mixed effects model in (B).



**Supplementary Figure 4. Behavioral effects of OFC modulation on light-avoidance.** (A) Violin plots displaying the latency to turn for saline-injected control mice (n=27, gray) and the C21- injected experimental mice (n=20, red), separated by age. Asterisks in (A) indicate statistical significance. \* $p < 0.05$ . Statistical test: two-way ANOVA.

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## **10 Curriculum vitae**

Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt

## 11 Affidavit

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe, insbesondere ohne entgeltliche Hilfe von Vermittlungs- und Beratungsdiensten, verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe. Das gilt insbesondere auch für alle Informationen aus Internetquellen.

Soweit beim Verfassen der Dissertation KI-basierte Tools („Chatbots“) verwendet wurden, versichere ich ausdrücklich, den daraus generierten Anteil deutlich kenntlich gemacht zu haben. Die „Stellungnahme des Präsidiums der Deutschen Forschungsgemeinschaft (DFG) zum Einfluss generativer Modelle für die Text- und Bilderstellung auf die Wissenschaften und das Förderhandeln der DFG“ aus September 2023 wurde dabei beachtet.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe. Ich erkläre mich damit einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

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Hamburg im April 2026

Henrik Østby