THE DOORS OF VISUAL PERCEPTION IN MICE.

by

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A DISSERTATION

Presented to the Department of Biology
and the Division of Graduate Studies of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

March 2023

DISSERTATION APPROVAL PAGE

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Title: The Doors of Visual Perception in Mice.

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Degree awarded March 2023

DISSERTATION ABSTRACT

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Doctor of Philosophy

Department of Biology

March 2023

Title: The Doors of Visual Perception in Mice.

A fundamental function of the brain is to generate subjective perceptual experiences, otherwise known as conscious awareness. However, in visual neuroscience, it is unclear why stimuli impacting the retina are only sometimes consciously perceived, other times going unseen (such as when viewing a very faint light at a distance, or during distracted driving). Further, it is uncertain which regions in the brain visual information must be routed to in order to enter conscious awareness. However, previous studies have suggested that variation in behavioral state factors such as attention, arousal, and motor activity can impact neural response dynamics and performance on visual tasks - suggesting a role for behavior state in determining the perceptual fate (conscious or unconscious) of visual stimuli. While most studies regarding neural mechanisms of consciousness are performed in human and non-human primates, the mouse is emerging as a model organism for the study of conscious awareness. This dissertation constitutes an exploration of the use of mice to study the neural correlates of visual perception. The literature review and experiments contained within are guided by three motivating questions: (1) Can the use of mice drive forward theories of consciousness developed in primates? (2) Does behavior state impact the neural response to near-threshold visual stimuli in mice? (3) Where

does conscious awareness enter along the visual processing hierarchy? Chapter 2 introduces the mouse as a model organism to study consciousness, chapter 3 describes results from an investigation into the role of locomotion and arousal on neural response thresholds, and chapter 4 summarizes results from a study on the role of the visual thalamus in directing selective visual attention. Amongst other findings, I demonstrate that mice are an ideal model organism for the study of consciousness, that behavior state impacts neural thresholds, and that activity in early visual regions is likely sufficient for conscious perception in mice.

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CHAPTER 1: INTRODUCTION

One of the most complex and mystifying functions of the brain is the ability to guard the 'doors of perception', otherwise known as conscious awareness. Over the years, poets, rockstars, and scientists alike have sought to understand the methods by which the brain generates perceptual awareness. But what exactly is consciousness? In neuroscience research, conscious awareness is often defined simply as subjective perceptual experience. For instance, a person with normal vision can become consciously aware of a red rose, whereas a person with blindness cannot not. However, even in normal vision, weak stimuli impacting the retina are only sometimes consciously perceived, and other times do not enter conscious awareness. For example, when driving at night, a very faint light coming from a distant headlight could go undetected for a relaxed 'veteran' driver, yet be detected by a 'new' driver anxious and alert from driving for the first time at night. It appears that, in everyday life your overall behavioral and arousal state can impact the detectability of stimuli that are near the threshold of perception.

Yet, it is uncertain which regions in the brain visual information must be routed to in order to enter conscious awareness. Visual processing starts in the retina, where photoreceptors detect light and transmit that information through the optic nerve to the thalamus, which then relays peripheral sensory information to the cortex. Once in the cortex, visual information undergoes further processing which takes place in distinct visual processing areas. Areas are hierarchical, meaning that "lower" visual areas perform less complex information processing (such as detecting the orientation of lines), and "higher" visual areas perform more complex visual processing (such as detecting faces). Decisions and motor output based on stimuli are thought to occur in the frontal cortex. But it is unknown where the shift from unconscious to conscious visual processing occurs along this processing hierarchy.

Leading theories of consciousness developed in primates fall into two general categories: those that place the neural correlates of consciousness at the back of the brain (early sensory regions), and those that place it at the front (higher order sensory areas and frontal-parietal network). While much progress has been made using primate models of consciousness, the causal testing of theories of consciousness is limited due to the many technical and ethical barriers inherent to primate research models. In contrast, for mice there are many powerful genetic tools for neural

manipulation and monitoring, and animal husbandry is much easier compared to primates. Chapter 2 of this dissertation introduces the mouse as a model organism to study consciousness, including the translation of human consciousness research to mice and vice versa.

Although stimuli impacting the retina are only sometimes consciously perceived (such as when viewing a very faint light at a distance), the detection of near-threshold visual stimuli is critical for guiding natural behavior. Consider that, while luminance levels can reach 100,000 lux at noon on a clear day, illumination at dusk can be as low as 1-5 lux, making both predator and prey difficult to detect. While studies in mice have shown that statistical noise-based fluctuations in baseline neuronal membrane voltage as well as population-level oscillatory rhythms[1,2] have some impact of the detection of visual stimuli, there are also striking correlations between behavioral state measures such as pupil diameter and locomotion activity and both visual activity and behavior[3–5]. For instance, pupil dilation is linked to task performance and firing rates in cortical sensory regions[2,6]. Further, motor activity in general has a widespread impact on neural activity in the cortex[5,7], and locomotion in particular shows a strong relationship with pupil diameter and amplitude of cortical visual response[8]. Yet, the visual functions of such behavioral state impacts on visual processing are unclear. One hypothesis is that motor signals that depend on behavior state factors, such as pupil dilation and locomotion, increase neural response gain, thereby enhancing stimulus processing during states of higher arousal and movement[9–11]. Such a process could potentially modulate the visual threshold based on behavioral context, enhancing detection of weak stimuli when they are behaviorally relevant, and/or temporally coincide with peaks in periodic oscillations in baseline arousal.

However, most existing studies of behavioral state impacts on cortical visual processing use salient stimuli (such as full-field stimuli), or have not continuously varied detectability near the threshold of visual detection. Thus, the effect of behavioral state on neural responses to near-threshold stimuli has not been well-explored. In chapter 3, I determined the impact of pupil diameter and locomotion state on population level neural responses to near-threshold visual stimuli. Using widefield calcium imaging and transgenic (GCaMP6s) mice, I monitored neural activity across the entire visual cortex simultaneously during passive viewing of near-threshold visual stimuli and concurrent recording of running speed and pupil diameter. The results shed light on the relationship between behavioral state and contrast sensitivity in visual cortex,

facilitating future casual studies to determine mechanisms that underlie the interaction between behavioral state and visual detection.

While many theories of visual consciousness stress the role of the visual cortex in generating visual perception, there are theories of consciousness that posit a role for the visual thalamus in generating conscious perception[12]. It is known that the pulvinar, a region of the visual thalamus, is in an ideal position to provide control over cortical information routing, as it has appropriate connectivity to relay information between cortical areas, and receives additional inputs that could regulate its function based on behavioral context. In chapter 4, using genetic tools for neuronal manipulation in mice, I report the efficacy of chemogenetics (DREADDs: designer receptors exclusively activated by designer drugs) to suppress pulvinar activity, in addition to establishing a behavioral model of visual attention in mice by adapting a selective visual attention task adapted from humans.

In summary, the work contained in this dissertation probes the neural correlates of consciousness using mice as a model organism and vision as a model modality. Three motivating questions guide this research: (1) Can the use of mice drive forward theories of consciousness developed in primates? (2) Does behavior state impact the neural response to near-threshold visual stimuli in mice? (3) Where does conscious awareness enter along the visual processing hierarchy? I approach these questions from three perspectives (1) the translation of human consciousness research to mice and vice versa, (2) the impact of behavior state on cortical responses to weak visual stimuli, (3) the role of thalamocortical pathways in routing visual information between cortical areas according to top-down, goal-oriented behavioral states. Chapter 1 introduces the mouse as a model organism to study consciousness, chapter 2 describes results from an investigation into the role of behavior state on neural response thresholds, and chapter 3 summarizes results from a study on the role of the visual thalamus in directing selective visual attention. Collectively, these chapters demonstrate that insights gleaned from the usage of mice can help solve one of neuroscience's fundamental puzzles: how the brain generates conscious awareness.

CHAPTER 2: THE MOUSE AS A MODEL FOR CONSCIOUSNESS

Abstract: Consciousness is arguably the most complex function of the brain, yet experimental models to study its neural mechanisms are limited due to technical and ethical barriers inherent to primate research models. However, the mouse is a promising animal model for performing causal studies to uncover the neural basis of conscious perception. In this review, I discuss the advantages and limitations of using mice as a model organism in consciousness research, highlight existing experimental paradigms in mouse research that dovetail with human studies of consciousness, and suggest future conceptual and experimental directions that can drive forward the study of consciousness using the mouse as a model organism.

Introduction.

Consciousness is simply subjective perceptual experience - it is the difference between a human experiencing the visual enjoyment of a beautiful sunset, and a camera experiencing nothing as it captures the image of that same sunset on film. While scientists have long argued that conscious experiences either arise from or are identical to processes in the brain, our understanding of the neural mechanisms that underlie consciousness - often referred to as the 'neural correlates of consciousness' (NCC) - is nascent, with only a few decades of scholarship directly addressing the matter. Most studies regarding neural correlates of consciousness are performed in human and non-human primates, and while much progress has been made using these methods, the testing of mechanistic theories is limited due to the many technical and ethical barriers inherent to primate research models.

In this review article, I curate evidence that the mouse is a promising new model organism for the study of neural mechanisms underlying consciousness. First, I give a broad overview of the field of consciousness research and knowledge that has been gained via primate studies. Then, I highlight existing studies in mouse research that provide insight into the NCC that would not be possible using primates. Finally, I suggest future conceptual and experimental directions that can drive forward the search for NCC's using the mouse as a model organism.

When looking for NCCs, it is important to bear in mind that consciousness itself has several different aspects to it. Frequently, a content-specific NCC is defined as the minimum

mechanisms jointly sufficient for any one specific conscious percept, whereas the full neural correlates of consciousness are the union of the sets of content-specific NCC for all possible contents of consciousness[13,14]. The percept can involve any kind of sensory experience, however, this review (and the field of consciousness research in general[15]) focuses mainly on the visual domain.

Neural Mechanisms of Consciousness: Knowledge from Primate Research.

Primate Study Methodologies. Identifying NCCs is made possible by relating behavioral correlates of consciousness (such as a verbal report of perception) to neural mechanisms that underlie them. Historically, visual neuroscience (and studies of consciousness) has primarily utilized humans, macaque monkeys, and cats, due to their large eyes (which facilitate ease of dissection, tracing, and recording) and the fact that the latter two share common features of human visual system (such as segregated parallel pathways in visual thalamus and ocular dominance orientation columns in visual cortex). Macaques in particular have a fovea and three cone pigments so they can be used to study high acuity trichromatic color vision. Studies of consciousness that utilize primate model organisms often employ electrophysiology, EEG, fMRI, and MEG and TCMS for recording and manipulation.

Comparing neural activity when a particular stimulus is perceived to neural activity when the identical stimuli is presented but not perceived (such as when viewing a near-threshold or bi-stable stimulus, or during forward or backward masking) is a common method of identifying potential NCCs. In experimental contexts, most often a report paradigm is used where the participant presses a keyboard button to report whether a stimulus was perceived or not. In contrast, no-report paradigms do not use an overt report such as pushing a button or giving a verbal answer, but instead rely on indirect methods to infer perception, such as using known correlations between pupil dilation state and reported visual detection.

Another way of searching for NCCs is to take a state-based approach, such as comparing brain activity in awake healthy participants performing no task to brain activity of subjects who are asleep, under anesthesia or have severely diminished consciousness such as in cases of coma or persistent vegetative state. However, the differences between brain states in these types of studies may entail more changes than just content of consciousness, but also greater overall

arousal, changes in attention, motor control, and other brain functions unrelated to consciousness. Additionally, it is important to distinguish between content-specific consciousness (the NCC) and underlying states of arousal and brain function which support content-specific consciousness. For instance, there are many midline brain structures responsible for unconscious processes such as breathing and heartbeat, which are all necessary for alert wakefulness, although not for perception of any specific conscious-content. Neuronal processes governed by these structures could be considered the 'background conditions' for the possibility of consciousness. Contrast said structures with the cerebellum for instance, without which a person is still conscious and has no disturbances of perception[16].

Candidate Regions for NCC in Primates: Cortex. There is a great deal of evidence that the specific contents of consciousness reside within the cortex[15]. Report based paradigms provide strong evidence for broad fronto-parietal network involvement in the conscious perception of stimuli[17–20]. However, while some no-report studies are in agreement with this[21,22], others suggest that at least some of the activity in this network belongs to processing the planning and execution of reporting during the task, leading some researchers to conclude that the true content specific NCC is located in posterior cortex only[23,24]. It is notable that the frontoparietal network reduces activity during loss of consciousness due to sleep, anesthesia, or vegetative state[25]. However, the fronto-parietal network also increases during seizures where patients experience unconsciousness[26]. During dreaming, activity in the prefrontal cortex is low compared to the awake state[27], and electrical brain stimulation during brain surgery shows that it's harder to elicit percepts through frontal stimulation compared to posterior cortical stimulation[28,29]. The results of lobotomy also suggest that frontal lobes are not required for consciousness. Thus, the role of the frontal cortex in generating content-specific consciousness is unclear, and there is still much up for debate regarding frontal versus anterior cortical involvement in the NCC[30].

There is also uncertainty surrounding the extent to which primary versus higher order sensory areas are involved in conscious perception. There are some visual stimuli known to elicit responses in V1 neurons absent conscious experience[31]. Similarly, it is sometimes possible to decode stimulus features (such as orientation) from V1 neurons even when the subject cannot discriminate the stimulus feature[32]. Single neuron recordings in monkeys performed during

binocular rivalry suggest that the activity of neurons higher up in the visual hierarchy corresponds to percept rather than stimulus[33].

In primates, the activity of neurons in layers 1-3 more often correlates with percepts than stimulus identity. Stimulus-evoked EEG activation shows a negative slow potential that originates in these supragranular layers and occurs between the onset of a stimulus and report of perception of a near-threshold stimulus, an effect which is abolished by anesthesia[34]. Further, the N1 ERP, which only occurs when a near-threshold stimulus is perceived, is thought to be due to excitatory input onto supragranular layers (possibly feedback from higher areas)[35].

Candidate Regions for NCC in Primates: Sub-Cortex. Brain stem injury usually leads to coma resulting from damage to reticular-activating and neuromodulatory systems[36]. However patients with severely damaged cortex yet functioning brain stem remain unconscious[37], suggesting that the brainstem alone is not sufficient for conscious experience. However, it is likely that the brain stem, hypothalamus, and basal forebrain (which themselves project to thalamus and cortex) provide the necessary background conditions to support consciousness. There is evidence that basal ganglia is required for alertness and emotional expression[38], but despite the many connections between cortex, basal ganglia, and thalamus[39,40], large bilateral basal ganglia lesions do not result in loss of consciousness[41].

Thalamic lesions can lead to coma, and thalamic stimulation can promote recovery in some patients with disorders of consciousness[42]. Higher order thalamic nuclei are of special interest because they project widely to the cortex and facilitate communication between cortical areas[43–46]. However, structural imaging studies in humans with disorders of consciousness show no relationship between thalamic atrophy and level of consciousness, but they do show a relationship between thalamic atrophy and the ability to communicate[47]. In macaque monkeys, the level of activity in the LGN does not correlate with conscious perception[48], but activity in the pulvinar does[49]. In humans under anesthesia, cortical function is more impaired than thalamic function[50]. It has also been shown that, during vivid sleep onset hallucinations, the thalamus is already deactivated for sleep[51].

The claustrum is a popular candidate NCC. Located beneath the insular cortex in primates, the claustrum is connected bidirectionally with many other brain regions, including cortex[52]. Notably, in a case report of a patient implanted with electrodes for surgery to treat

epilepsy, stimulation of the white matter tract beneath the claustrum induced loss of perceptual consciousness with no other discernible impact, although this early finding has not always been replicated clinically[53,54].

Theories of Consciousness in Primates. There are several leading theories of consciousness which have been developed using primate models, and I will only touch on a few of the major ones here (for a more extensive review see Northoff & Lamme 2020[55] or Seth & Bayne 2022[56]).

Global Neuronal Workspace Theory. The Global Workspace Theory (GWT) of consciousness claims that brain processes become conscious when information is broadcast within a 'global workspace' of interconnected brain networks. According to this theory, fronto-parietal networks play the role of a central hub that coordinates information broadcasting to 'local processors' in the service of the organism's goals[57]. Also key to the GWT is the finding that, in primates presented with near-threshold visual stimuli, brain activity in anterior cortical regions 'ignites' (amplifies greatly) around 200–300 ms after stimulus onset only when the stimulus is perceived[15].

Integrated Information Theory. The Integrated Information Theory (IIT) of consciousness puts forth that neural differentiation and integration are key signatures of conscious brain processing[58]. For instance, during states of unconsciousness such as deep dreamless sleep, anesthesia, and certain kinds of seizures, neural activity is highly synchronized and undifferentiated. Neural integration is the idea that there is some minimum number of neural processes that must occur in coordination in order for perception to occur, and indeed neural integration decreases and modularity of brain processes increases during sleep[13]. Neither integration nor differentiation alone can be used as a measure of consciousness however, as integration can increase under anesthesia or during certain kinds of seizures[13]. Proponents of the IIT have developed a measure of conscious awareness called the perturbational complexity index (PCI), which is obtained by using EEG to capture the degree of spatiotemporal differential of brain activity in response to perturbation (such as TCMS stimulation, or a light shined in the eyes), and is a useful prognosis index for the return of consciousness in patients in a vegetative state. It is important to note however that PCI values being a good indicator of consciousness is not incompatible with other theories of consciousness.

Recurrent Processing Theory. Recurrent Processing Theories of consciousness assert that higher to lower order feedback processing is a key mechanism for generating conscious perception. In primates, there is evidence that while a near-threshold stimulus triggers an initial feed forward flow of stimulus-evoked activity throughout primary and higher order cortical areas (which takes around 120-140 ms), it will not be perceived unless a feed backward flow of information from higher to lower cortices occurs following the feedforward flow[59]. There is evidence to support this in neuroimaging studies of brain damaged patients[60].

The Mouse: A Promising Candidate Model Organism for Studying Consciousness.

While decades of primate studies have led to the development of several leading theories of consciousness, the development of genetic tools for neural manipulation at the circuit level has lagged in primates compared to other model organisms such as the mouse. However, in recent years mice have emerged as a popular model organism within the field of visual neuroscience, and their use as a model organism to study consciousness will allow causal testing of leading theories of consciousness that were developed within the primate literature.

Experimental Tools in Mouse Research. The current state of knowledge in consciousness research shows that, in addition to electrophysiology, most primate studies of consciousness utilize EEG, MEG and fMRI. With the use of mice as a model organism to study consciousness come several advantages: (1) the mouse brain is smaller, therefore more of the brain can be recorded from simultaneously even with electrophysiology, (2) the use of transgenic mouse lines allows many ways to record neurons at both the single cell and population level, (3) genetic tools for neural manipulation facilitates testing of causal theories of neural mechanisms for consciousness, and (4) mouse colony maintenance and husbandry is considerably easier compared to primates.

For recordings of neural activity, mouse research relies heavily on single cell and extracellular electrophysiology, single cell and neuronal ensemble-level calcium recordings using 2-photon microscopy, and large-scale population-level recordings using widefield calcium imaging. There are many transgenic mouse lines developed for targeted recordings of specific cell types and brain regions using genetically encoded calcium indicators. As will be discussed

below, there is also some pioneering usage of fMRI and EEG emerging in mouse studies. In mouse research, electrode technology has been rapidly advancing, with electrodes containing up to 1000 recording sites on a 1 cm shank, and the ability to record hundreds of neurons per shank.

For manipulation of neural activity in mice, in addition to electrolytic lesions and electrical stimulation via electrodes, usage of opto- and chemo-genetics is common. In optogenetics, neuronal ion channels are genetically engineered to contain light-sensitive proteins, such that when genes for these channels are expressed by the neurons, researchers can then control neural activity (excitation or suppression) using light. Chemogenetics is similar, but instead of using light sensitive ion channels, channels (either excitatory or inhibitory) that are sensitive to an otherwise biologically inert ligand are used, and said ligand is injected into the animal (a reversible process). There are many transgenic mouse lines developed for targeting manipulation of specific cell types and brain regions using opto- and chemo-genetics.

The Mouse Visual System: Retina, Midbrain, and Cortex. The mouse visual system shares many of the same basic properties as observed in primates, as well as certain differences that are worth noting. Mice are nocturnal. Nevertheless, they rely on vision under ecological conditions[61–64]. Compared to primates, the mouse retina is rod-dominated, with only 3% of mouse photoreceptors being cones. Mouse cone photoreceptors only detect short and medium wavelength light (no red photopigment receptor). In primates such as macaques and humans, cone cells are most densely located at the fovea, and a main purpose of eye movements is to shift the fovea so that it aligns with incoming light such that important features of the environment are detected with the greatest visual resolution. In mice, there is no fovea. Further, while mouse retina is more densely packed with photoreceptors than macaque, the smaller size of their eye results in fewer photoreceptors per area of visual space - thus mouse vision is about 200 times poorer in acuity than human vision (20/2000)[65,66].

Mice have similar types of retinal ganglion cells as primates, however there is a greater diversity of said cell types in mice compared to primates. Like primates, mouse retinal ganglion cells have center-surround organization, plus other types of receptive fields (such as direction selectivity) that may or may not be present in primates (not fully established yet). Like primates, mouse LGN receptive fields exhibit classical on and off center-surround responses, and the size

of receptive fields in mouse LGN is consistent with the receptive field sizes of mouse retinal ganglion cells[65,66].

As seen in primates, mouse primary visual cortex consists of a 6-layered structure with retinotopic organization and a variety of inhibitory and excitatory cells. Neurons in mouse V1 exhibit spatial frequency tuning and orientation selectivity. However, there is no 'pinwheel' structure to the layout of orientation selectivity tuning (a feature which all rodents lack). Yet, the degree of orientation selectivity observed in mouse V1 is very similar to what is seen in humans, prompting some to suggest it might reflect a fundamental aspect of cortical computation, with the pinwheel pattern observed in primates playing a role in wiring rather than coding efficiency[66]. Similar to primates, regions of the mouse visual cortex are organized hierarchically at both an anatomical and functional level, with functional connectivity mirroring anatomical hierarchy[67]. In addition to classical measures of processing hierarchy such as response latency, receptive-field size, phase-locking to stimuli and response decay timescale, the correlation between neural activity and behavioral choice also increases along the cortical hierarchy of the mouse visual system[67].

Eye Movements. Recently, there has been increasing use of lightweight eye tracking options for studying eye movements in mice under freely moving conditions, which is ideal because head-fixation limits eye movements in mice (who rely heavily on the angle of their head to direct their line of sight). In foveate animals, eye movements often serve the purpose of shifting the visual gaze such that light from the most salient features of the world falls on the fovea. Such eye movements are usually conjugate (track together). However, mice are afoveate, with bilaterally placed eyes. Eye movements in the mice have been observed to be either non-conjugate (occur in opposite directions) and perform a role of stabilization of the visual gaze in response to jitter from head movement, or they follow head movements and are related to image stabilization with respect to the ground (saccade-and-fixation without a fovea)[68].

Visual Attention. Primate studies of consciousness make frequent use of visual attention paradigms. Recently, it has been demonstrated conclusively that mice exhibit visual selective attention, by adapting canonical visual attention tasks (Posner-style cueing task, cue vs. no cue, filter task) in human research for mice[69]. Mouse performance on these tasks was consistent with

primates, paving the way to use mice to study the genetic and neuronal circuit mechanisms of selective attention. Indeed, mouse studies probing the impact of spatial attention on brain activity have shown that selective attention enhances visual response to salient regions of visual space, while suppressing visual response to irrelevant visual information, which is also observed in primates[70]. It is thought that in mice, selective attention improves the decoding of visual information compared to other information (such as motor)[71]. Interestingly, one study found that motor variables can activate visual networks without interfering with perceptual outputs, and may support perceptual stability by solving a "self-other" disambiguation problem during sensory-motor interactions in the environment[71]. Regardless, work such as this demonstrates that mice display fundamental signatures of visual spatial attention spanning behavioral, network, cellular, and synaptic levels.

Pupil Dynamics. Similar to primates, pupil dilation can be used as a coarse measure of attention and arousal in mice. Even under anesthesia, pupil dilation correlates with brain wide state fluctuations[72]. Pupil dilation increases with locomotion, and changes in pupil diameter area are associated with performance in selective visual attention tasks in a Yerkes-Dodson inverted U-curve[71]. In a study using rats which is easily adaptable to mice, pupillometry was used alongside fMRI and calcium imaging to reveal a global brain network of activity that correlates with pupil diameter size[72]. These studies in mice are congruent with human literature examining pupil dynamics and visual performance[73].

Near-Threshold Stimulus Paradigms: Behavior Tasks for Studying Consciousness in Mice. Mice can also be trained to perform a wide array of visual tasks, some of which are quite analogous to the kinds of visual studies canonically used in primate studies of consciousness. For instance, mice can be trained to report whether or not they observe a near-threshold stimuli, (presented near either the absolute detection threshold or the change detection threshold), so that brain activity during conscious processing and non-conscious processing can be compared. As with primates, you can quantify aspects of mouse performance on visual tasks using the usual measures such as response accuracy, reaction time, perceptual sensitivity and decision criteria.

For example, in a study by Busse et al.[74], operant conditioning was used to train mice to detect the presence or absence of grated lines in a two alternative forced choice (2AFC) task.

This involved a 3-port nose poking protocol wherein the central port triggers the start of the trial, the stimulus (drifting gratings) is presented on either the left or the right side, and the mouse reports which side the stimulus appeared by poking the corresponding choice port. As in most mouse behavior studies, correct choices were rewarded with water and incorrect choices were discouraged with a 'timeout' during which no further trials could be initiated at the central port. Mice were first trained on full contrast stimuli, then the contrast of the gratings were varied such that psychophysical curves for contrast sensitivity could be obtained. For a task such as this, training typically lasts 4 weeks, and consists of several stages of learning. Like primates, mice can perform hundreds of trials a day in this task. Using these methods, Busse et al obtained a contrast threshold of 10%, falling in the middle of a large range of published values at the time.

In another study aimed at measuring the absolute contrast detection threshold, Histed et al.[75], trained mice to manipulate a lightweight lever while head fixed in a yes-no task in order to report the presence or absence of full field gratings. Contrast was varied at a single, highly visible grating. Mice initiate the trial by depressing the lever, after which the stimulus was displayed at a random time, and mice could report detection of the stimulus by releasing the lever. This task takes around 60 days for water-restricted mice to learn, which is long for typical mouse behavioral experiments but similar to the amount of time required to train a nonhuman primate on a near-threshold lever press detection task. Again, mice can perform hundreds of trials a session with reliable performance across sessions. The minimum detectable contrast level obtained was 2%, at a spatial frequency of 0.1 cpd, with the values of contrast thresholds being reliable across animals. The researchers suggested that the nature of the task (i.e., optomotor, water maze, 2AFC, yes/no) and stimulus (i.e., size of stimulus) used to measure contrast sensitivity likely impacts the precision of the measured threshold values, and could explain differences in contrast detection threshold measures across studies.

A study by Long et al.[76] probed the role of task design in impacting measures of perceptual sensitivity. In this study, mice were trained to discriminate between two orthogonal orientations in two separate task designs - 2AFC and go/no-go. The goal of the study was to determine the effect of contrast on orientation discrimination. The 2AFC task utilized nose ports, and the target stimulus was a vertical (non-target stimulus was horizontal) static sinusoidal grating. For the go/no-go task, mice were head fixed, the stimulus was a drifting sinusoidal grating, and mice indicated whether the stimulus was horizontal or vertical by licking. The

method of reporting observations is not trivial; licking and lever pressing activate different brain regions - an important consideration for paired neural recordings, which they also performed during these tasks. The results of the study showed that, for both task designs, both performance and the neural response difference between preferred and orthogonal orientations increases with increasing contrast, but the go-no/go task was more vulnerable to shifting response criteria if more than one stimulus contrast was introduced.

The future of near-threshold stimulus paradigms in mice might rely heavily on touchscreens embedded into freely moving arenas, such as in a study by You and Mysore[77], where they showed that mice can perform a touchscreen-based 2AFC visual task. In this behavioral task, freely behaving mice were placed in a plexiglass tube within a soundproof operant chamber containing a touch-sensitive screen covered in a plexiglass sheet except for three holes where the mouse was able to interact with the touchscreen via nose-touch. Rewards were delivered at the opposite face of the box from where the touch-screen was located. A central nose poke initiated each trial, and mice indicated which side a stimulus appeared on by poking the left and right nose holes. Importantly, this task takes advantage of the highly exploratory nature of natural mouse behavior. Using these methods, the researchers discovered that mice can report targets as brief as 100 ms. Further, varying the duration of stimulus presentation allowed the testing of visual short term memory performance in mice.

Candidate Regions for NCC in Mice: Cortex. Considering the available tools for neural manipulation, current state of knowledge regarding brain function in the mouse, and analogous behavioral and experimental paradigms for studying visual behavior in mice - which regions are the best candidates for playing a potential role in neural mechanisms of consciousness? There are a number of existing studies in mice exploring the role of cortical and sub-cortical regions in supporting consciousness.

A study by Glickfield et al.[78] used the behavioral paradigm developed in 2012 by Histed et al.[75] to provide evidence that V1 plays a role in determining the threshold of detection. In this study, mice were trained to perform change detection tasks reporting changes in either the contrast or orientation of visual stimuli. Using optogenetics, researchers reversibly inhibited V1, and found that suppression of V1 increases the threshold for detecting changes in both orientation and contrast of visual stimuli. If V1 is required for detection of near-threshold stimuli

as this study suggests, that would be particularly aligned with posterior hot zone theories of consciousness. However, it was noted that even when PV interneurons were stimulated at a level that produced maximal effects, visual perception was not entirely eliminated.

A study by Gale et al.[79] also provides evidence that early visual cortex could play a role in generating consciousness percepts. In this study, mice were trained to report the location of a grating that was backward masked within a 50 ms window from target stimulus offset. The neural mechanisms underlying the phenomena of backward masking are not fully understood. To test the necessity of visual cortex for backward masking, the researchers optogenetically silenced mask-evoked cortical activity in V1 during the task, which fully restored performance on the visual task. These results suggest that mask stimulus processing within V1 plays a role in explaining how a masking stimulus can inhibit the perception of a target stimulus that came before it. This study was the first time a robust backward masking paradigm was developed in mice, paving the way for mice to be developed further as a model organism for studying consciousness.

Further investigations into the role of mouse visual cortex in conscious perception were reported in a study by Jin and Glickfield[80]. While it had been known that stimulus orientation and contrast are encoded across the entire mouse visual cortex, Jin and Glickfield[80] used a used a go/no-go contrast and orientation detection task to test the necessity of V1 and three higher order visual areas (LM, AL, PM) for perception of orientation and contrast. They found that suppression of V1, LM or AL decreased performance on the task by increasing perceptual thresholds, whereas suppression of PM selectively increased false alarm rates without impacting performance. Their results suggest that primary and more lateral visual areas may be more responsible for perception of orientation and contrast, whereas more medial and anterior regions of mouse visual cortex may instead play a role in regulating noise during decision-making. The authors noted that it was interesting to find functional specialization for perception of features that are broadly represented across visual areas, as neurons in PM are similarly responsive to task stimuli, orientation tuned, and suppressed by activation of inhibitory interneurons as V1, LM and AL are. These results highlight the critical importance of performing causal, not just correlational, studies to determine the NCC.

A study which looked at visual areas outside of the visual cortex in mice for correlates of visual perception was performed by Zatka-Haas et al.[81] In this study, head-fixed mice were

trained to discriminate changes in contrast and orientation of a grated patch and reported their perceptions by turning a wheel. Concurrent with the task, widefield calcium imaging was used to perform large-scale calcium recordings. The researchers found task-related activity in multiple cortical areas: visual, secondary motor, primary motor and somatosensory. They then performed causal testing by performing optogenetic inactivation of each cortical region individually, and found that inactivation only impaired performance when executed in visual and motor regions. However, it was unclear whether the motor cortex actually played a role in visual discrimination, or just a role in reporting. To address this question, the researchers used a neurometric model to relate the animal's choices on the task to its brain activity during the task, and found that activity in visual regions promotes contra-versive choices and suppresses ipsi-versive choices, whereas motor areas promote both contra- and ipsi-versive choices. The same model predicted the effect of local optogenetic inactivation. These results show that visual discrimination is supported through visual regions but not primary motor areas.

A study Resulaj et al.[82] investigated the time course of visual perception as well as what regions and layers of visual cortex are involved. During a head-fixed visual detection task that requires visual cortex, the researchers used optogenetics to silence stimulus evoked activity at varying intervals post-stimulus presentation. They discovered that the threshold duration of activity in visual cortex for perceptual discrimination in this task is between 40 and 80 milliseconds, and the earliest spiking activity occurred in layer 4 of V1. Surprisingly, during this time window the vast majority of neurons responding to the stimulus only fire one or no spikes, and less than 16% of neurons fire more than two spikes. These findings suggest that the very first few visually evoked spikes in visual cortex are sufficient to enable a perceptual decision, and provide further support for the hypothesis that early visual regions play a role in conscious perception in mice.

The holy grail of finding content-specific NCC is finding a region that, when lesioned, results in the loss of a specific percept, and when activated, triggers the percept, independent of stimulus. A study by Marshel et al.[83] achieved the latter goal in an experiment using red-shifted channelrhodopsin (ChRmine) combined with multiphoton-holography (MultiSLM). ChRmine is a recently identified opsin that produces large photocurrents with millisecond spike-timing fidelity, and is compatible with simultaneous two-photon Ca2+ imaging. Using ChRmine together with holographic devices allows the creation of arbitrarily specified light patterns and

millisecond-precision control. In this study, mice were first trained on an orientation discrimination task. Then, neurons responsive to either horizontal or vertical lines were identified across cortical layers. Finally, ChRime optogenetics and MultiSLM were used to stimulate neurons that were previously activated by either horizontal or vertical lines. Strikingly, this recreated the original neural activity and task performance, suggesting that mice experience optogenetic stimuli as true percepts. They also found that, during optogenetic activation of orientation-selective ensembles, neuronal activity asymmetrically propagated from layer 2/3 to layer 5, and smaller layer 5 ensembles were as effective as larger layer 2/3 ensembles in eliciting orientation discrimination behavior, suggesting that cortical layer–specific dynamics should be taken into consideration during the search for NCCs in mice. In line with the study by Resulaj et al.[82], they also found that across subjects, only a few tens of neurons were required for recruiting a robust enough population response to drive high discrimination performance, because network amplification mechanisms carry signals outside of V1 to drive specific behavior.

While many studies of consciousness focus on phenomenal consciousness - the subjective experience of seeing a grated line, for instance, another aspect of consciousness is the felt sense locating one's "center of subjectivity" within the body. This aspect of consciousness can perhaps best be understood through its negation - the dissociative experience, sometimes referred to as an 'out of body experience'. People who report experiencing dissociation as a result of dissociative drugs (such as ketamine), spiritual experience, or brain disorder, often report the existence of a dissociative state as being central to the experience of altered or expanded conscious awareness. Behaviorally, dissociation is apparent in the dissociation of stimulus detection from stimulus-related affective responses, or when the sense-of-self is dissociated from body position or action. Vesuna et al.[84] developed a model for studying dissociation in mice by administering ketamine or phenylcyclidine to mice and performing large-scale imaging of neural activity via widefield and 2-photon calcium imaging. To behaviorally measure dissociation between sensory perception and affective response, the researchers used the reflexive paw-flick as a measure of sensory detection and paw-licking as a measure of affective/self-protective response to the aversive stimuli. Calcium imaging showed that the dissociate agents induced a 1-3 Hz oscillatory rhythm in layer 5 neurons of the retrosplenial cortex. Further electrophysiological recordings performed after drug administration showed a rhythmic coupling of activity in retrosplenial cortex and connected thalamic regions, but

uncoupling from most other brain regions, most notably an inverse correlation between retrosplenial cortex and frontally projecting thalamic nuclei. To causally test whether layer 5 retrosplenial neurons play a role in dissociation, they used optogenetics to rhythmically activate said neurons, which recapitulated dissociation-like behavioral effects in mice. These results were consistent with a human clinical study they also performed which showed that in a patient with focal epilepsy, pre-seizure self-reported dissociation correlated with a similarly localized rhythm in the homologous deep posteromedial cortex, and local brief electrical stimulation of this region elicited dissociative experiences. Thus, by establishing a mouse model of dissociation, researchers were able to identify a cortical rhythm that underlies states of dissociation, and causally determine the role of a specific cortical layer and region in generating the dissociative rhythm and behaviors.

Candidate Regions for NCC in Mice: Sub-Cortex. Recently, the claustrum has come into focus as a structure that could potentially be involved in the NCC[85,86]. Similar to higher order thalamic nuclei, the claustrum has reciprocal connectivity with the cortex, as well as a number of other brain areas[87]. The claustrum receives inputs primarily from frontal regions of cortex, but projects back to sensory modality-related cortical regions of both frontal and sensory cortex. Regions of cortex that share direct cortico-cortical connections also share inputs from the same area of claustrum (sometimes from single claustrum neurons with bifurcating axons). A mouse study by Wang et al.[57] showed that projections from claustrum to cortex are topographically organized and form sensory modality-specific modules with different regions of cortex[88]. Beyond the cortex, the claustrum receives one-way input from several subcortical structures, such as the amygdala, hippocampus, and thalamus (including limbic nuclei). This broad connectivity alone suggests a role in 'higher order processes' such as conscious perception. A study using resting-state fMRI in awake rats[75] suggests that the claustrum plays an important role in the rodent homolog of the primate salience network. In primates, the salience network is identified as strong functional connectivity between the cingulate cortex (controlling attention) and the insula (processing valence). However, Smith et al.[40] showed that rodent cingulate cortex is anatomically and functionally connected to the claustrum rather than the insula. Further, the claustrum receives neuromodulatory input from basal forebrain cholinergic centers. Such

neuromodulatory inputs could act globally across different claustrum sub-regions and enhance or suppress excitability of large networks of claustrum outputs.

Considering the sensory connections of the claustrum, as well as it's input from limbic structures, the claustrum seems ideally situated to integrate information about value with sensory information, which could in turn be used to direct activity in cingulate cortex in order to guide attention. This could be achieved via claustrum activating cortical interneurons, causing feed-forward inhibition and the suppression of cortical processing of irrelevant stimuli, a theory supported by a mouse study using optogenetics by Jackson et al[89]. Another mouse study supports this; McBride et al.[90] performed a study in awake mice using optogenetics and neuropixels recordings from over 15,000 neurons in which they found that brief stimulation of the claustrum affected many inhibitory but few excitatory neurons in the cortex, with more inhibition occurring in frontal regions and deeper layers, and more excitation occurring in posterior regions and superficial layers. According to this theory, the claustrum may play a role in consciousness by acting as a hub for associating sensory and limbic information to influence attention via cortical inhibition. Behavioral evidence in support of this theory exists in a mouse study by Atlan et al. which used a task where animals learn to track a visual cue to identify the correct response. In this study, an auditory distractor paired with the visual cue did not decrease performance on the task unless the claustrum was also inactivated[91].

There is also evidence for a role for the claustrum in another aspect of consciousness, wakefulness vs. sleep. A mouse study by Narikiyo et al.[92] showed that local field potentials evoked by claustrum stimulation resembled cortical down-states associated with slow wave sleep. Further, lesioning claustrum reduced slow wave activity in the cingulate cortex during deep sleep, suggesting a role for the claustrum in governing aspects of cortical activity during sleep via feedforward inhibition.

Theories of Consciousness Applied to Mice. What can existing mouse research contribute towards the investigation of current leading theories of consciousness, which go beyond mere description of regions involved?

Global Neuronal Workspace Theory. In humans, spontaneous brain activity displays signature spatial temporal organization during wakeful awareness as compared to during deep sleep, anesthesia, or coma. However, until recently it was unclear whether there is evidence for

such organization in mice. By adapting a human protocol for fMRI mapping to head-fixed mice, researchers probed the functional network topography and temporal dynamics of spontaneous brain activity in awake mice and compared their results to similar studies performed in anesthetized mice[93]. They observed that during the awake state, brain networks are functionally organized such as to maximize inter-area information transfer, which is not present in the anatomical axonal connectome. They also reported that fMRI activity in awake mice exhibits unique spatiotemporal dynamics that are characterized by a state-dependent participation of arousal-related forebrain nuclei. Their results suggest that spontaneous brain activity supporting consciousness in awake mice is shaped by state-specific involvement of basal forebrain arousal systems.

Integrated Information Theory. Due to its value in translating results from mouse to human research, studies involving the use of EEG and fMRI in mice are growing. In the field of consciousness research, a recent study by Claar et al.[94] performed EEG recordings in mice then applied perturbational complexity analysis - a method of predicting the presence of consciousness in humans - to assess consciousness in mice. Perturbational complexity analysis involves stimulating the brain with a brief pulse of electricity, recording the response using EEG, and characterizing the spatiotemporal complexity of said response. In humans, when consciousness is low, such as during coma or deep sleep, so is perturbational complexity. In this study, the use of a mouse model allowed for a deeper examination of the neural circuits underlying perturbational complexity by probing the cellular level. Researchers used neuropixel probes to stimulate the cortex and recorded both high-density EEG and spiking activity throughout the cortico-thalamo-cortical system under conditions of wakefulness and anesthesia. The results of this study showed not only that perturbational complexity is lower during anesthesia than wakefulness in mice, but also that thalamic bursting is necessary for the late component in the evoked EEG believed to be associated with consciousness in humans. These findings are consistent with the theory that cortico-thalamo-cortical interactions contribute to ongoing neural activity supporting consciousness. Further, the perturbational complexity index was developed by proponents of the integrated information theory of consciousness. This study lends further support that PCI is a good measure of consciousness, and validates that this method can be used in mice to identify conscious and unconscious states (as well as the transitions between them).

Recurrent Processing Theory. Considering the prominence of theories purporting that both feed-forward and feedback processing are necessary for conscious experience, Kitazono et al[95] decided to test the hypothesis that the subnetworks underlying consciousness are recurrent. Their analysis of the whole-brain mouse connectome identified 'cores' with strong bidirectional connections consisting of regions of the neocortex, thalamus, and claustrum. There were no cores identified that included regions thought to be unrelated to consciousness (such as cerebellum). These results are consistent with recurrent processing theories of consciousness.

Future Directions for Studying Consciousness with Mice.

Experiments. In the future, one of the main benefits that will be gained from using mice as a model organism to study consciousness is the ability to causally test the leading theories of consciousness that have been developed in primate models. While the number of possible mouse experiments to test theories of consciousness is vast, there are a couple of ideal types of experiments to perform first.

To further probe the plausibility of the recurrent processing theory of consciousness, experimenters could utilize opto- or chemo-genetics to selectively inhibit feedback input between interconnected areas (for instance from higher to lower order visual areas, or from visual cortex to pulvinar, or from cortex to claustrum) during a near-threshold visual discrimination task. An important aspect of testing recurrent processing theories of consciousness is to disambiguate the more general role for recurrent processing in unconscious brain operations from any potential role that is specific to supporting consciousness.

An important disagreement between the global workspace and integrated information theories of consciousness involves the potential necessity of frontoparietal networks in conscious processing of visual stimuli. An important genre of experiment to perform in mice would be to selectively inhibit frontoparietal networks during a near threshold visual task. One way of doing this without interfering with the mouse's ability to report its perceptions during the task would be to have a delay period between stimulus offset and behavioral report, and to suppress frontoparietal networks only during that delay period. Knowledge of whether or not mice can perform visual discrimination tasks without frontoparietal network activation would give a definitive answer as to whether or not global neuronal workspace theory as it currently stands is

a plausible theory of consciousness. While such a study would not test integrated information theory directly, it would address a component of the IIT - the assertion that early sensory processing alone can be a signature of consciousness (i.e., the posterior hot zone theory).

Overcoming Potential Limitations of Studying Consciousness Using Mice. Considering the debate surrounding whether frontoparietal networks play a role in conscious processing per se or rather just the behavioral report of what was consciously perceived, the question of whether or not no-report paradigms can be performed in mice looms large. However, there are a few potential ways that no-report paradigms could be performed with mice.

One potential way that no-report paradigms could be performed with mice would be to develop the use of pupil and eye tracking to determine whether a stimulus was perceived. While there are some challenges to this, such as the fact that pupil states track general arousal, which is highly linked to but not identical to perception, there are studies in mice showing that pupil diameter is predictive of performance on visual discrimination tasks[2]. Using pupil diameter to detect perception sans report in mice could be achieved by "training" mice to associate a visual stimulus (preferably salient/of ecological relevance) with a water reward, and then interleaving brief or low contrast (near threshold) versions of the stimuli (with either no reward or random chance of reward being received). If pupil diameter is a reliable indicator of performance/task response, it could be used as a proxy for perception in a no-report near threshold stimulus paradigm designed to compare conscious and unconscious processing of identical stimuli in mice.

Another potential no-report paradigm would be to use a behavioral task that relies weakly on visual short term memory, such as a near-threshold discrimination task wherein a mouse must wait a few seconds in between stimulus detection and report (via nose poke, licking, lever, wheel turn, etc.). To ensure that performance on such a task is a measure of visual detection, not memory, the duration of delay between stimulus offset and report should be optimized so as to not tax visual short term memory too much, yet still allow perception and motor planning/output to be temporally disambiguated.

Finally, the use of ethologically relevant stimuli and behavior tasks could be of service in developing no-report paradigms in mice. For instance, consider that mice are extremely sensitive to looming stimuli (they even have a looming response in retinal ganglion cells) because hawks

are natural predators to mice. Thus, the response criterion for mice to respond to looming stimuli is presumably very low. Researchers could take advantage of the reliability of mouse responses to such ethologically relevant stimuli by presenting them (e.g., looming stimuli) near the perceptual threshold in a no-report behavioral paradigm, such as one that utilizes pupil dilation or locomotion as an indicator of stimulus perception. This type of study could be performed either head-fixed or in an open arena with the use of lightweight brain monitoring systems, depending on how much control is desired over animal movement. While the use of looming stimuli would likely evoke affective responses that could confound identification with content-specific NCCs, when combined with other methods of identifying NCCs, the advantages of having a no-report paradigm may outweigh the drawback of having to figure out how to disambiguate neural processing related to affective and phenomenal states of consciousness.

Another concern sometimes raised about the use of mice to study consciousness is the question of whether or not mice in fact experience consciousness. Due to the inherently private nature of first-person knowledge of experience, it is true that it cannot be known for sure whether or not mice are conscious, just as it cannot be known for sure whether or not Donald Trump is conscious. But, humans have language, and because other people may verbally communicate the contents of their conscious experience, it makes it easier to take a leap of faith and presume that they in fact have conscious experiences too (although in experimental contexts, reaction times and 'presses of a button' are more precise measurements of stimulus detection than verbal report).

Devastatingly, the ability to communicate the existence of one's conscious state and the contents of one's conscious perceptions is severely impaired or totally destroyed in patients with 'locked-in' syndrome[96], a condition where, whether it be due to traumatic injury or organic disease, a person is entirely or nearly entirely paralyzed but their conscious awareness remains totally or partially intact. Because clinical tests of consciousness are behavioral due to the neural correlates of consciousness not being well-understood, people with locked-in-syndrome can remain consciously aware yet in emotional and physical discomfort for decades. The condition is usually discovered by a caretaker years into the onset of what was misdiagnosed as a vegetative state. In a way, the main limitation of studying consciousness in mice is the same as the main

challenge to identifying consciousness in locked-in patients: no ability to communicate one's perceptions using language.

Conclusion.

Mice can perform many of the canonical visual tasks used in human studies of consciousness, and they have many shared features of brain organization, information processing, and behavior execution as primates. While mice are not "little primates", mechanistic insights gleaned from their usage can have a large potential clinical benefit for humans, in addition to solving one of neuroscience's fundamental puzzles: how the brain generates conscious awareness.

CHAPTER 3: THE IMPACT OF BEHAVIOR STATE ON VISUAL PROCESSING OF NEAR-THRESHOLD STIMULI

Abstract: The detection of near-threshold visual stimuli is critical for guiding natural behavior. While luminance levels can reach 100,000 lux at noon on a clear day, illumination at dusk can be as low as 1-5 lux, making both predator and prey difficult to detect. While it is known that quantity of light impacts visual detection, it is not known to what extent an animal's behavioral state impacts its neural sensitivity to a given quantity of light. Previous studies have shown that variation in behavioral state factors such as attention, arousal, and motor activity impact neural activity and performance on visual tasks. However, whether such behavioral state factors can impact neural responses to near-threshold stimuli has not been well-explored. In this study, I determined the impact of pupil diameter and locomotion state on population level neural responses to near-threshold visual stimuli. Using GCaMP6s mice and widefield calcium imaging, I monitored neural activity across the entire visual cortex simultaneously during passive viewing of near-threshold visual stimuli and concurrent recording of running speed and pupil diameter. I found that increases in pupil diameter and running speed are associated with a decrease in the neural response threshold and an increase in contrast sensitivity across visual areas. The results shed light on the relationship between behavioral state and contrast sensitivity in visual cortex, facilitating future casual studies to determine mechanisms that underlie the interaction between behavioral state and visual detection.

Introduction.

The ability to detect weak or brief stimuli is critical in ethological conditions. For example, predators and prey move quickly under low contrast conditions such as during dawn, dusk, and night. Importantly, visual thresholds also fluctuate over time and based on behavioral context - for instance, attentional engagement can enhance performance on visual tasks in both primates and rodents[69,97]. This raises a question: which extra-retinal signals impact whether a near-threshold stimulus is detected?

While studies have shown that statistical noise-based fluctuations in baseline neuronal membrane voltage as well as population-level oscillatory rhythms[1,2] have some impact of the

detection of visual stimuli, there are also striking correlations between behavioral state measures such as pupil diameter and locomotion activity and both visual activity and behavior[3–5]. For instance, pupil dilation is linked to task performance and firing rates in cortical sensory regions[2,6]. Further, motor activity in general has a widespread impact on neural activity in the cortex[5,7], and locomotion in particular shows a strong relationship with pupil diameter and amplitude of cortical visual response[8]. Yet, the visual functions of such behavioral state impacts on visual processing are unclear. One hypothesis is that motor signals that depend on behavior state factors (such as pupil dilation and locomotion) increase neural response gain, thereby enhancing stimulus processing during states of higher arousal and movement[9–11]. Such a process could potentially modulate visual thresholds based on behavioral context, enhancing detection of weak stimuli when they are behaviorally relevant, and/or temporally coincide with peaks in periodic oscillations in baseline arousal. However, most existing studies of behavioral state impacts on cortical visual processing use salient stimuli (such as full-field stimuli), or have not continuously varied detectability near the threshold of visual detection. Thus, the effect of behavioral state on neural responses to near-threshold stimuli has not been well-explored.

In order to determine behavioral state impacts on fluctuations in neural responses near the visual threshold, I performed widefield calcium imaging in GCaMP6s mice during passive viewing of near-threshold stimuli and concurrent recording of pupil diameter and running speed. The use of mesoscale imaging allows for the comparison of neural responses to a single stimulus across all cortical visual areas simultaneously, which could reveal whether visual areas may be adapted for different behavioral roles with different thresholds and state-dependence. Further, while state-dependence in vision has focused on V1, models of conscious perception predict different responsiveness along the visual hierarchy depending on whether a stimulus is consciously perceived. The results demonstrate that behavioral state modulates sensitivity to weak stimuli across cortical visual areas. This work sheds light on the relationship between behavioral state and contrast sensitivity in visual cortex, and lays the foundation for future casual studies to determine mechanisms that underlie the interaction between behavioral state and visual detection.

Results.

In a cohort of 7 mice, I performed widefield calcium imaging of the visual cortex during passive viewing of near-threshold stimuli. Simultaneously, I recorded running speed and pupil diameter. Mice were head fixed yet able to choose to stand still or locomote “in place” on a Styrofoam ball. Visual regions and points of analysis were determined via retinotopic mapping and pixel-wise activation patterns (**Fig. 3.1D**).

Experiment 1. In order to determine which stimulus parameters to use for the rest of the study (i.e., to identify a stimulus duration that is likely perceptible at higher contrasts but not at lower contrasts) I first presented mice with a wide range of stimulus conditions, wherein the longest duration is likely visible (260 ms, which is in the range of mouse behavioral response on visual detection tasks) and the shortest duration is most likely invisible (16 ms) (**Fig. 3.1A, B, C**). Stimulus contrasts followed a log scale between zero and 100 percent. In the first set of parameter-testing experiments (**Table A**), I observed two groups of neural responses to increasing contrast, with a noticeable difference in activity between them (266 & 133 ms versus 66, 33, and 16 ms) (**Fig. 3.2A, B, C**). To gain further resolution in the intermediate parameter range, I performed a second set of parameter-testing experiments using stimulus durations between 66 and 116 ms.

***Table A*:** Summary of Data Collected (n = recording sessions)

MOUSE ID	Experiment 1	Experiment 2	Experiment 3
305RT	n = 3	n = 3	n = 3
305LT	n = 3	n = 3	n = 3
124LT	n = 3	n = 3	n = 3
2DLT			n = 3
2GRT			n = 3
2GLT			n = 3
2ERT			n = 3

Experiment 2. In this second set of parameter testing experiments (**Table A**), I observed a smooth gradation of contrast response functions (**Fig. 3.2D**). I chose to perform the rest of the experiments in this study using stimuli displayed for 100 ms, which is towards the middle of this parameter range and evokes a dynamic range of neural responses across contrasts.

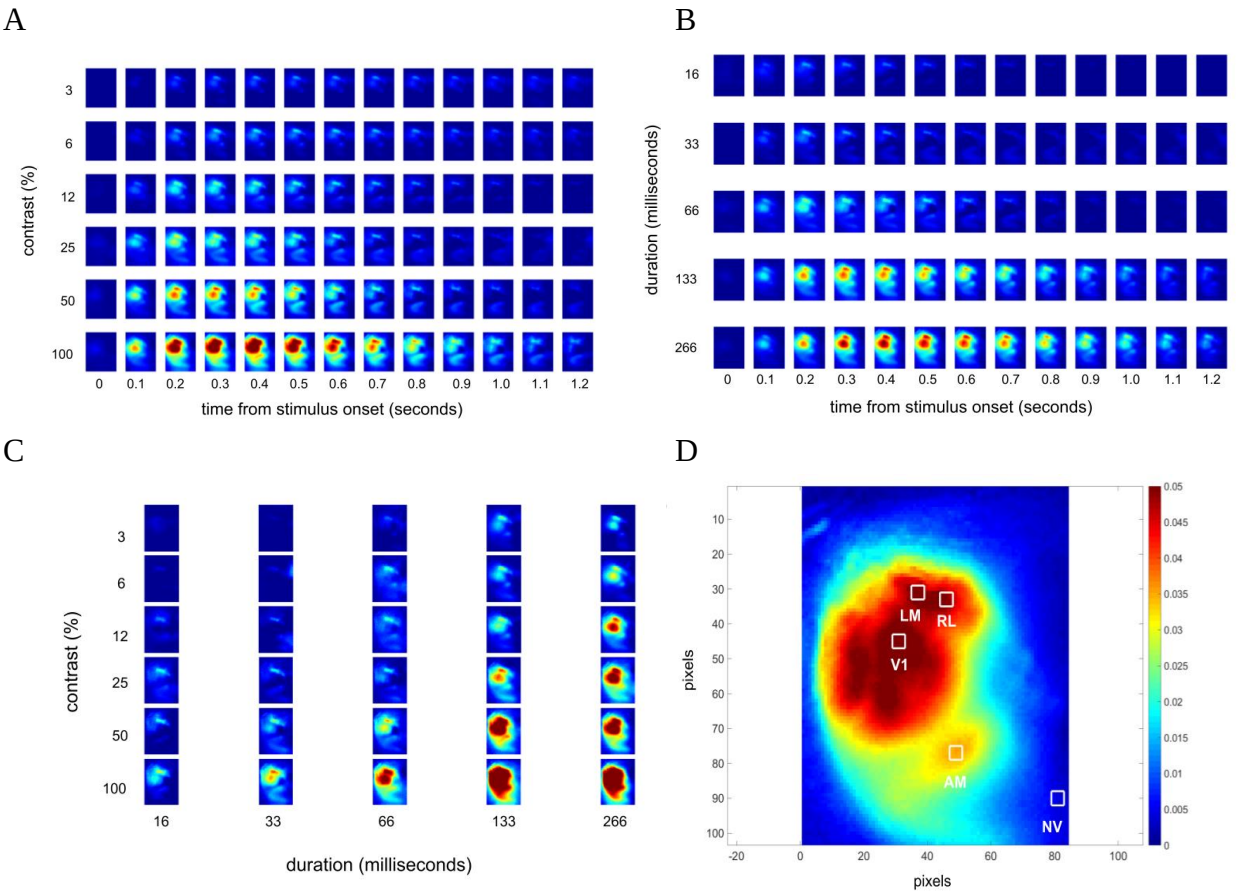


Figure 3.1*: Stimuli displayed for brief durations across a range of contrasts evoke a dynamic range of neural responses*. **(A)** Example (n = 1 mouse, n = 1 session) peri-stimulus cortical activation for each contrast condition (averaged over all durations) in experiment 1. **(B)** Peri-stimulus cortical activation for each duration condition (averaged across contrasts) in experiment 1. **(C)** Peak response to each stimulus condition in experiment 1 (n = 1 mouse, n = 1 session). **(D)** Points used for analysis of region-specific responses.

Experiment 3. In order to determine whether running modulates visual response to near-threshold stimuli, I performed a final set of experiments (**Table A**) using the stimulus parameters selected in the previous experiment (duration = 100 ms, contrast = 0, 3, 6.25, 12.5, 25, 50, 100%). To compare neural response functions on running versus stationary trials, it is necessary to have a large enough and, ideally, similar number of trials that fall into the 'stationary' versus 'running' category during each recording session. Because the stimulus paradigm consists of seven

conditions with an average trial duration of around 4 seconds, during each recording session I was able to obtain many trials for each stimulus condition and, in most cases, a similar number of running and stationary trials.

Behavior State During Recording. **Fig. 3.3A** shows concurrent recordings of locomotion speed and pupil diameter over time for one example mouse and recording session. Generally, single session running and pupil traces showed changes in pupil diameter that occur more gradually and occupy a larger variety of values compared to changes in locomotion speed, which were often characterized by periods of stationarity punctuated by periods of high running speed. Across all recording sessions, most of the trials fall into one of two categories: a state of low pupil diameter and low locomotion speed, and a state of high pupil diameter and high locomotion speed (**Fig. 3.3B**).

The frequency distribution of trial-averaged locomotion speeds pooled across all recording sessions (all mice, recording sessions, and stimulus conditions) shows that on roughly one third of trials mice were stationary, while during the rest of the trials mice were locomoting to some degree, with a second frequency peak towards the upper range of locomotion speeds (around 6.5 cm/sec) (**Fig. 3.3C**). To categorize trials into low versus high locomotion states, a threshold of 1 centimeter per second running speed was set based on previous work by our lab[8]**.** Locomotion speeds below 1 cm/sec are usually either stationary or small stabilizing movements, while above this threshold mice are observed to be running at some speed. Therefore, 1 cm/sec was used as a categorical threshold between low and high locomotion states.

The frequency distribution for pupil diameter size across all trials pooled across all recording sessions and trial conditions shows that across all trials mice displayed large and small pupil diameter sizes in similar proportions, with two frequency peaks around 0.25 a.u. and 0.65 a.u., respectively (**Fig. 3.3D**). Note that, in order to account for (albeit small) variations in pupil anatomy and the distance and angle of eye camera placement, pupil diameter sizes were normalized so that a pupil diameter of 1 corresponds to the 99.5^{th} percentile of pupil diameter sizes, and a few outlier trials fall above 1 a.u. In order to categorize trials into high versus low pupil diameter state, a threshold value of 0.4 a.u. was set based on the valley between peaks of the pupil diameter distribution shown in **Fig. 3.3D**.

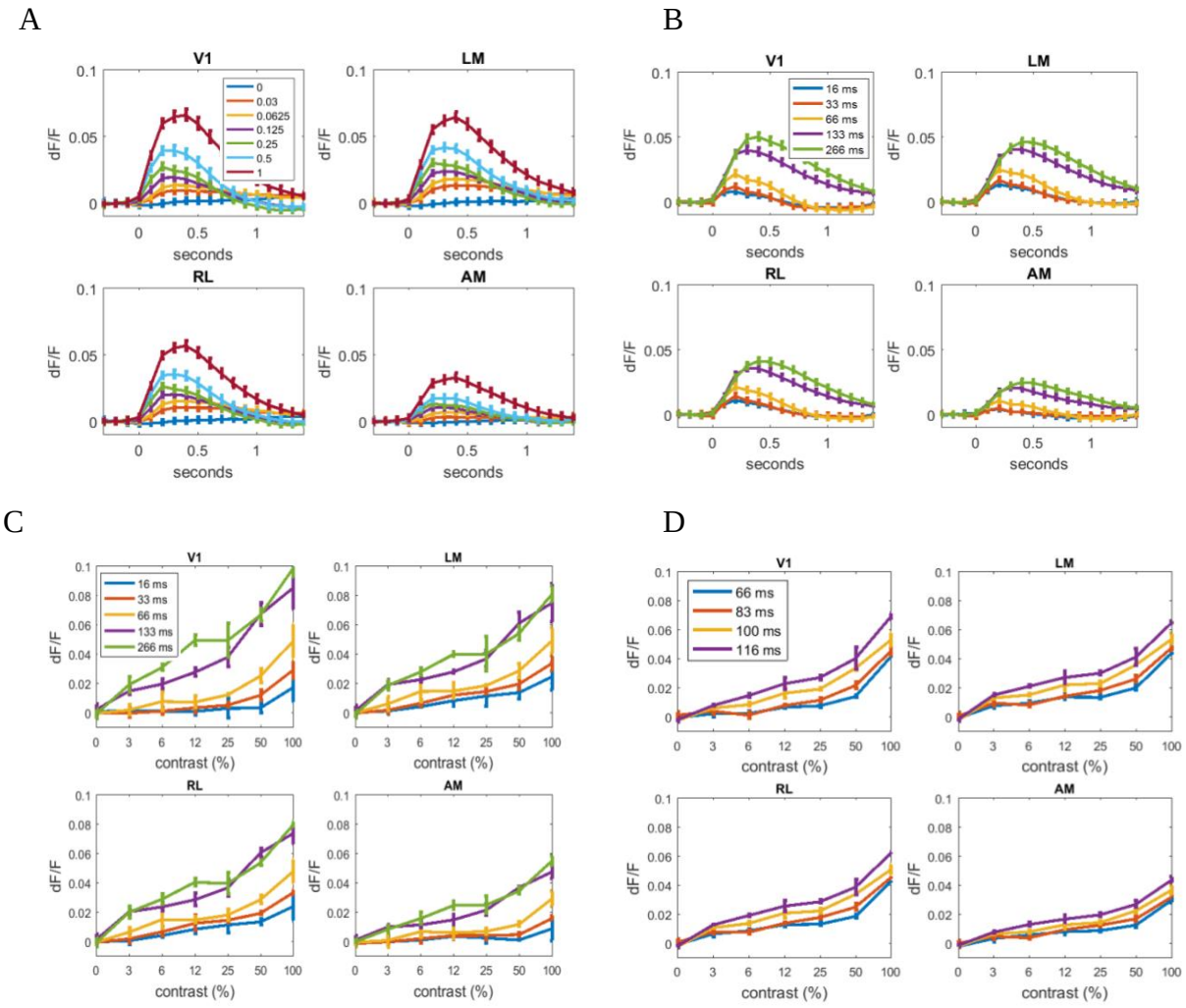


Figure 3.2*: Peri-stimulus and contrast response functions for a range of durations*. **(A)** Example (n = 1 mouse, n = 1 session, standard error is across trials) region-specific (V1, LM, RL, AM) peri-stimulus response to varying contrast (averaged across durations) in experiment 1. **(B)** Region-specific peri-stimulus response (n = 1 mouse, n = 1 session, standard error is across trials) to varying duration (averaged across contrasts) in experiment 1. **(C)** Region-specific contrast response functions for each duration (n = 3 mice, n = 9 sessions, standard error across mice) in experiment 1. **(D)** Region-specific contrast response functions for each duration (n = 3 mice, n = 11 sessions, standard error across mice) in experiment 2.

The Impact of Behavior State on Neural Threshold. By categorizing trials as low versus high behavior states, I was able to compare behavior state-specific neural contrast response across visual areas (**Fig. 3.4A-D, Fig. 3.5A-D**). **Fig. 3.5A** shows contrast response functions separated by locomotion state, and **Fig. 3.5B** shows contrast response functions separated by pupil diameter state. These figures illustrate that, while neural response increases with contrast across all behavior conditions, there is a greater neural response at all contrasts during high behavior states. Contrast sensitivity appears to decrease in general from lower to higher visual areas. The

impact of behavior state on contrast sensitivity is qualitatively similar when trials are separated by locomotion state versus pupil diameter state.

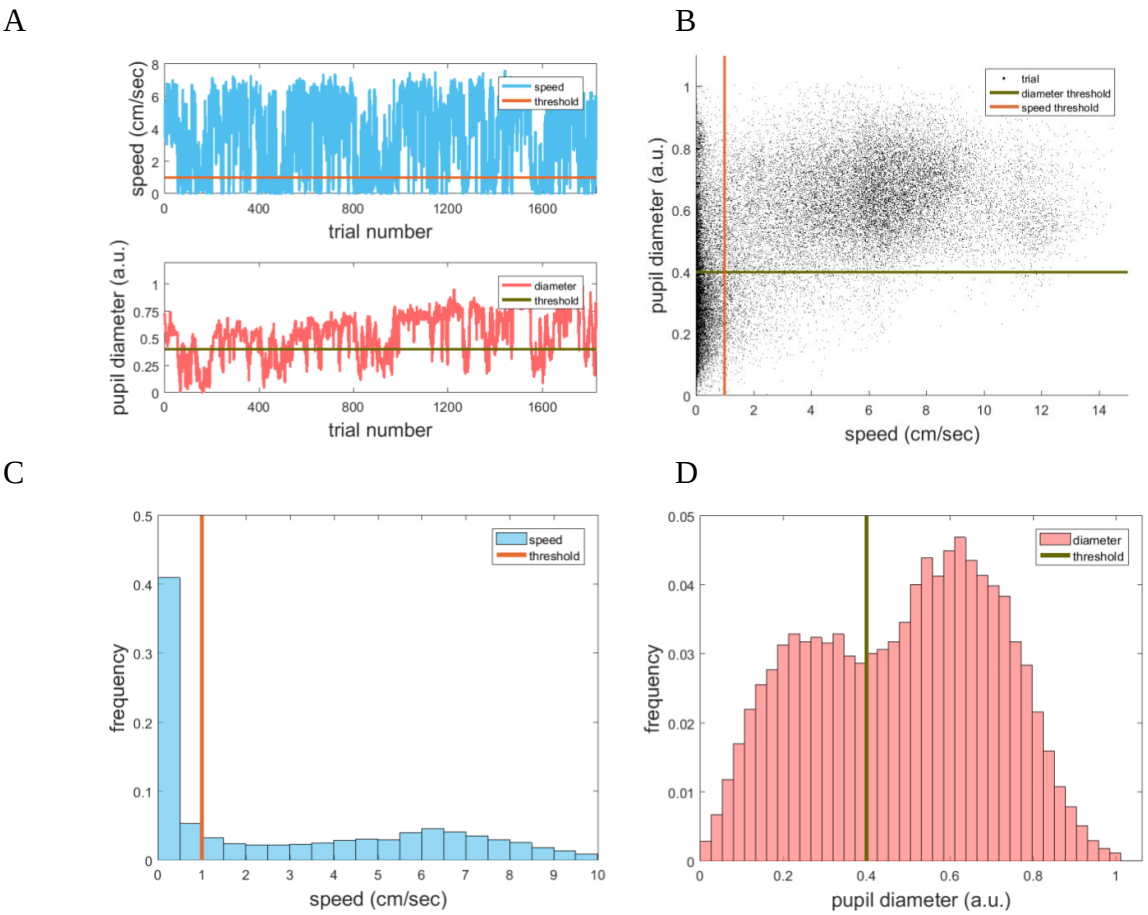


***Figure 3.3**: Two prominent behavior states emerge during passive viewing*. **(A)** (Top) Locomotion speed over time for one example mouse and recording session in experiment 3, (bottom) normalized pupil diameter over time for the same mouse and recording session. **(B)** Pupil diameter and speed of locomotion for each trial, across all mice and recording sessions (N = 7 mice, N = 3 recording sessions per mouse (21 sessions total) N = 38,340 trials across all recording sessions, mean number of trials per recording session = 1823 trials, SD = 411 trials). **(C)** Distribution of trial-averaged locomotion speed across all mice, recording sessions, and trials. **(D)** Distribution of trial-averaged pupil sizes across all mice, recording sessions, and trials.

By obtaining the contrast response functions for each behavior state, I was able to calculate the behavior state specific neural threshold for contrast at this brief duration, for each visual area (**Fig. 3.5C, D**). Neural threshold was defined using the common C50 metric, which is defined as the stimulus parameter value at which the neural response reaches half of its maximum value (which in this case is when the stimulus is at 100% contrast). C50s for each state and visual area are shown in **Fig. 3.5C** and **3.5D**, separated by locomotion speed and pupil size, respectively.

For each visual area, a single sample t-test with Bonferroni correction for multiple comparisons was performed to test for a statistical difference between the average neural threshold (C50) value for the high behavior state and the average neural threshold for the low behavior state. Rejection of the null hypothesis in all visual areas, whether trials were separated by pupil or locomotor state, demonstrates that the neural threshold for contrast decreases when locomotion and arousal increase (**Fig 3.5C, D**). Scaled region and state-specific contrast response functions also show changes in the shape of response curve between low and high states of movement and arousal (**Fig. 3.6A, B**).

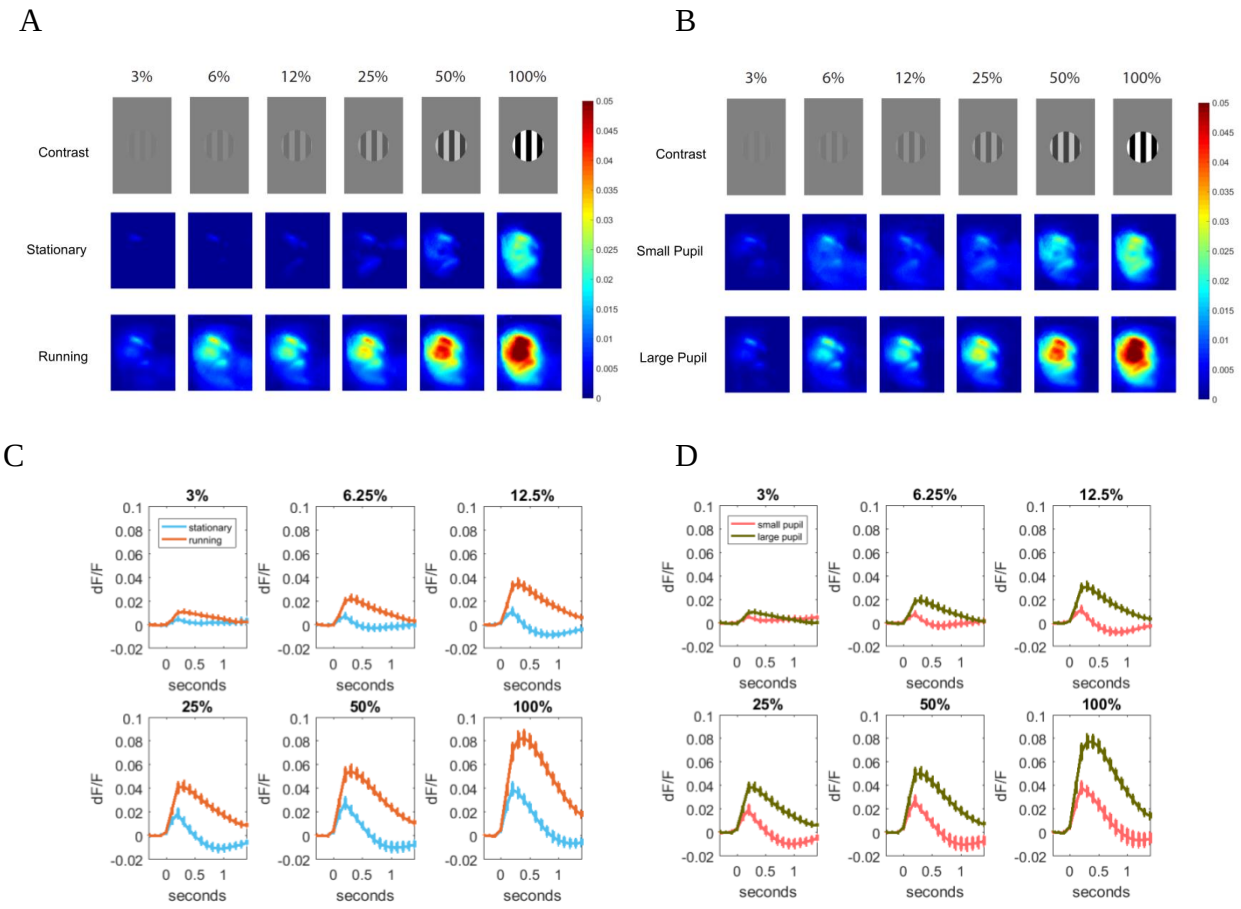


***Figure 3.4**: State-specific neural response to varying contrast*. **(A)** Peak pixel-wise cortical activation for each stimulus condition in experiment 3 (top row), separated by locomotion state (middle row: stationary, bottom row: running), for one example mouse. **(B)** Peak pixel-wise cortical activation for each stimulus condition (top row), separated by pupil state (middle row: high pupil diameter, bottom row: low pupil diameter), for one example mouse. **(C)** Region-specific peri-stimulus response to each stimulus condition, separated by locomotion state, for one example mouse. X-axis is time since stimulus presentation. **(D)** Region-specific peri-stimulus response to each stimulus condition, separated by pupil state, for one example mouse. X-axis is time since stimulus presentation.

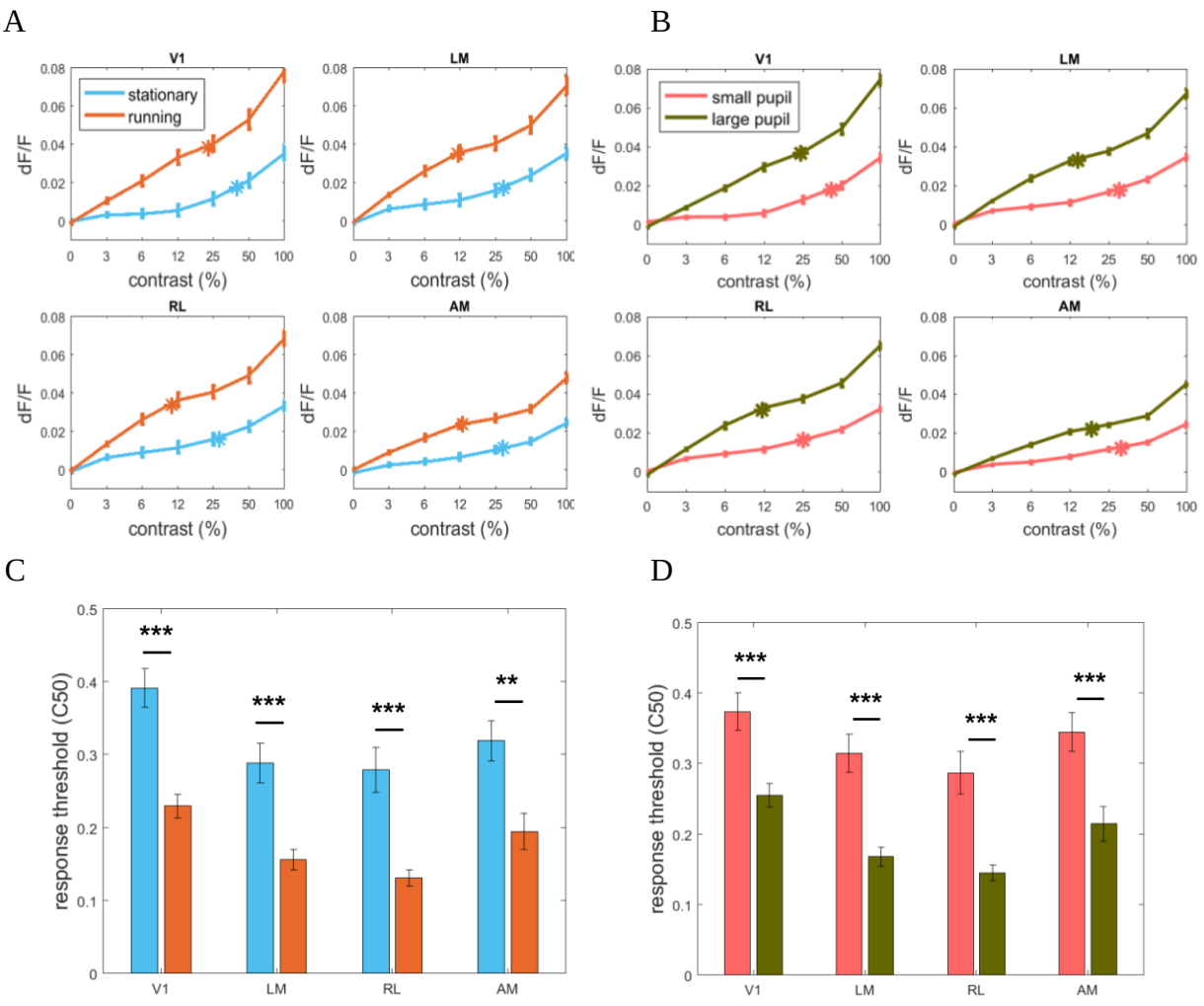


Figure 3.5*: Contrast response functions and neural thresholds separated by behavior state*. **(A)** Region and locomotion state-specific contrast response functions across all mice and recording sessions, (n = 7 mice, mean number of trials per condition across all sessions = 5273, SD = 207). Error bars represent the standard error of the mean across mice. The neural threshold (C50 value) of each response function is marked with an asterisk. **(B)** Region and pupil state-specific contrast response functions across all mice and recording sessions. Error bars represent the standard error of the mean across mice. The neural threshold (C50 value) of each response function is marked with an asterisk. **(C)** Mean neural contrast threshold (C50) during low versus high locomotion states, across visual areas. Error bars show standard error of the mean across mice (n = 7). Statistically significant differences in C50 value denoted as p<0.05 (*), p<0.01 (**), p<0.001 (***). **(D)** Mean neural contrast threshold (C50) during low versus high pupil diameter states across visual areas. Error bars show standard error of the mean across mice (n = 7). Statistically significant differences in C50 value denoted as p<0.05 (*), p<0.01 (**), p<0.001 (***).

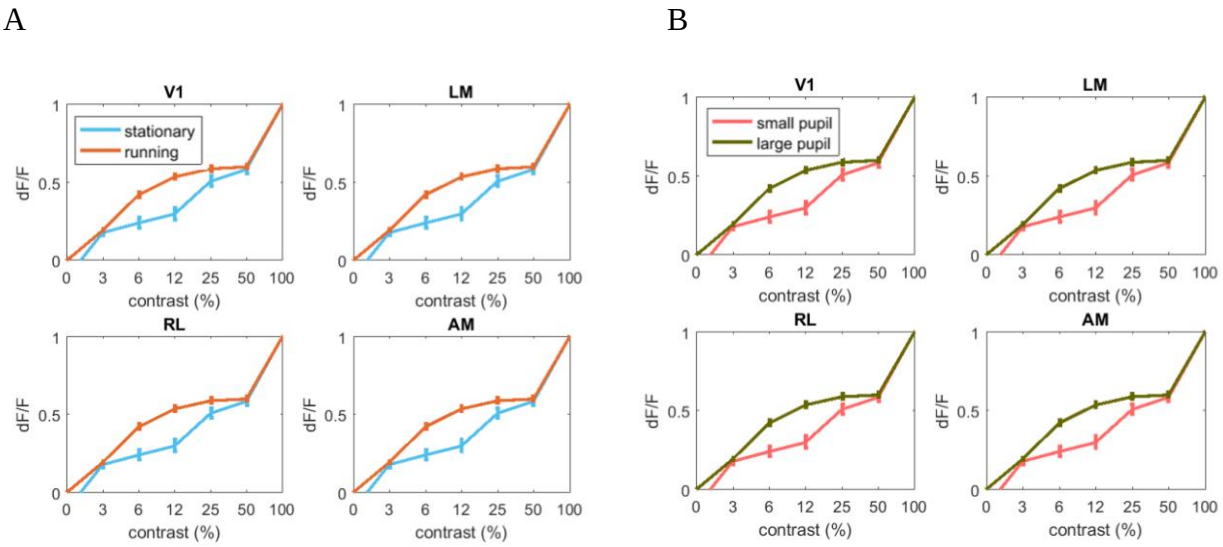


***Figure 3.6**: Scaled region and locomotion state-specific contrast response functions.* **(A)** Region and locomotion state-specific scaled contrast response functions across all mice and recording sessions, (n = 7 mice, n = 21 recording sessions, mean number of trials per condition across all sessions = 5273, SD = 207). Error bars represent the standard error of the mean of the across mice (n=7). **(B)** Region and pupil diameter state-specific scaled contrast response functions across all mice and recording sessions, (n = 7 mice, n = 21 recording sessions, mean number of trials per condition across all sessions = 5273, SD = 207). Error bars represent the standard error of the mean of the across mice (n=7).

In summary, I found that: **(1)** During passive viewing of weak visual stimuli mouse behavior states mostly fell into one of two categories: high pupil size during running, or low pupil size while stationary (**Fig. 3.3B**). **(2)** Contrast sensitivity decreases moving from lower to higher visual areas (**Fig. 3.5A, B**). **(3)** The neural threshold for contrast decreases when locomotion and arousal increase (**Fig. 3.5C, D**) and **(4)** Behavior state alters the shape of the neural contrast response function (**Fig. 3.6A, B**).

Discussion.

There are several strengths of the present study. Firstly, while the investigation of state-dependence in vision has focused primarily on V1, in this study I measured the impact of behavioral state across both primary and higher order visual areas, finding no state-dependent differences in visual response at the population level across visual areas. Secondly, in contrast to most existing studies of behavioral state impacts on cortical visual processing, the present study used weak visual stimuli and continuously varied detectability near the threshold of visual

detection, finding that the neural threshold for contrast decreases when locomotion and arousal increase. Thirdly, because mice in this study were engaged in passive viewing, changes in locomotion and pupil diameter can be assumed to be due to changes in the internal dynamics of brain states, rather than motivated by task demands, suggesting that the impact of behavior on the neural threshold observed in this study is due to spontaneous fluctuations in arousal and movement. A prominent limitation of this study is the lack of causal testing of any proposed mechanism to account for the observed impact of behavior state on neural threshold. However, this work lays the foundation for future causal studies to determine mechanisms that underlie the interaction between behavioral state and near-threshold visual detection. The results demonstrate that behavioral state has an impact on the neural response to near-threshold stimuli, which is congruent with the hypothesis that behavior state modulates neural response gain in order to improve the detectability of weak stimuli under natural conditions such as at dusk or dawn.

Follow-up investigations to the present study could examine the role of potential mechanisms underlying the relationship between behavioral state and visual response thresholds. For instance, recent studies in mice have shown that weak visual stimuli are often not detected if slow oscillatory activity is pronounced in the cortex, and that slow oscillatory activity in the cortex often coincides with small pupil diameter states[2,70]. It is also possible that the behavior-state-dependent changes in neural sensitivity to contrast observed in this study are the result of gain control feedback originating from frontal motor control or higher order visual regions[98]. Additionally, locomotion is known to impact neural responses in subcortical structures that project to cortex such as the thalamus[99], and the pulvinar, a higher-order thalamic region, has a known role in modulating contrast sensitivity[100]. Finally, broadly-projecting neuromodulatory pathways such as those originating from cholinergic and noradrenergic brainstem nuclei are known to play a role in global arousal state[101,102].

Of further interest is a recent study which found that neural activity in anterior visual regions is suppressed rather than enhanced by locomotion[103]. In contrast, the present study found that neural activity increases across all visual areas as locomotion speed and pupil diameter increase. However, Christensen & Pillow[103] recorded neural activity using laminar-specific GCaMP expression and 2-photon calcium imaging at the cell ensemble level in layers 2-5, whereas I recorded population level calcium activity using widefield imaging of superficial visual cortex. Considering that some studies have found that the strongest driver of L1 neurons is

locomotion[104], the search for mechanisms underlying the impact of behavior state on neural thresholds may benefit from future studies performing laminar-specific characterization of neural responses to near-threshold stimuli.

Interestingly, while existing literature shows an inverted-U shaped relationship between pupil diameter and performance on sensory detection tasks[2], the present study found that the neural threshold decreases as pupil diameter increases, even at the largest pupil diameter values. The existing literature also presents evidence that locomotion impairs the detection of weak visual stimuli[2], whereas I found that increased locomotion speed corresponds with decreased neural thresholds even at the highest running speeds. The apparent conflict between the existing literature and the present study's findings brings into question the relationship between neural and perceptual thresholds. An ideal future study would be to train mice to perform a visual detection task using near-threshold stimuli and perform widefield calcium imaging of the entire dorsal visual cortex simultaneously with recording of pupil diameter and running speed. Results from this hypothetical study would indicate whether the effect of behavioral state on neural response thresholds observed in the present study reflect changes in reports of visual detection.

In conclusion, the results from this study demonstrate that behavioral state modulates sensitivity to weak stimuli across cortical visual areas, shedding light on the relationship between behavioral state and contrast sensitivity in visual cortex, and laying the foundation for future casual studies to determine mechanisms that underlie the interaction between behavioral state and visual detection.

Methods.

Animal use. All procedures were conducted in accordance with the ethical guidelines of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee at the University of Oregon. Adult mice 3–6 mo old, both male and female, were used in this study. Animals were maintained on a 12-h light/12-h dark reverse light cycle. Experiments were performed during the dark phase of the cycle.

Transgenic mouse line. I used a transgenic mouse line which expresses the fluorescent calcium indicator GCaMP6s in excitatory neurons throughout the cortex under the control of a

tetracycline-controlled transactivator (tTA) driver line[105]. Because I measured neural responses to very weak stimuli, I used the slower but more sensitive variant of gcamp (GCaMP6s). The use of this transgenic line allows broad expression of the indicator and provides a global view of cortical activity.

Surgical procedures. Animals were anesthetized with isoflurane (3% induction, 1.5%–2% maintenance, in O2) and body temperature was maintained at 37.5°C using a feedback-controlled heating pad. Fascia was cleared from the surface of the skull following scalp incision and a custom steel headplate containing a circular well was attached to the skull using Vetbond (3M) and dental acrylic. The headplate well was centered over V1 (2.5–3 mm lateral of the midline and 1 mm anterior of Lambda). Carprofen (10 mg/kg) and lactated Ringer's solution were administered subcutaneously, and animals were monitored for three days following surgery.

Measuring Locomotion. Mice were head-fixed above a spherical treadmill and locomotion was measured via an optical mouse placed on the side of the spherical treadmill. Mice were able to choose to stand still or "locomote in place".

Measuring Pupil Diameter. Pupil recordings were performed using a DALSA genie M1280 IR camera and the 'image acquisition tools library' from MATLAB. The camera was placed just outside the lateral edge of the monitor so as to not obscure it, about 20 cm away from the contralateral eye. Acquisition occurred at a rate of 10 Hz. To track pupil diameter across trials, I used supervised machine learning (DeepLabCut) to perform automatic labeling of pupil perimeter points, and a custom python script to calculate the longest axis of the pupil diameter in each eye tracking video frame. In order to account for (albeit small) variations in pupil anatomy and the distance and angle of eye camera placement, pupil diameter sizes were normalized so that a pupil diameter of 1 corresponds to the 99.5^{th} percentile of pupil diameter sizes, and a few outlier trials fall above 1 a.u.

Stimuli. Visual stimuli consisted of 30 degree 0.16 cpd grated patches and were presented at a variety of durations across experiments, ranging from 16 - 260 ms, with 100 ms being the duration of all stimuli during the third and main set of experiments. The inter-trial-interval was

semi-randomized with a mean duration of 2.5 seconds. Stimulus contrasts followed a log scale between zero and 100 percent. Stimuli were generated in MATLAB using the Psychophysics toolbox extensions[106,107], and presented on gamma-corrected LED monitors oriented tangentially 20–25 cm from the contralateral eye. Stimuli were presented on the monitor at a rate of 60 Hz.

Widefield Imaging. A widefield microscope (Scimedia, Inc.) equipped with a sCMOS camera (PCO, 10 Hz acquisition) was used to measure GCaMP6s signal though the skull during blue LED excitation (Luxeon Rebel 470 nm, 0.1 mW/mm2 at the sample). Every four frames, a green LED (Luxeon Rebel 530 nm, 0.1 mW/mm2 at the sample) was used for excitation in order to measure hemodynamic signals, which were subtracted from the blue frames[105]. To obtain boundaries of visual areas, visual areas were first mapped using a topographic stimulus consisting of a bar of 1/f noise sweeping in either azimuth or elevation, and the amplitude and phase of the Fourier component of the ΔF/F signal were calculated at the stimulus frequency (0.1 Hz), which were later used to align sessions across animals[108]. In order to compare contrast sensitivity across visual regions, "points of peak activation" within four areas were identified by overlaying the borders obtained via retinotopic mapping on a pixel-wise map of the mean neural activation during peak stimulus response. For each pixel chosen to represent the peak activation of a given visual area, peak activation was calculated within a 25 pixel squared region centered on the peak activation pixel.

Data Analysis. For each animal, a central point corresponding to the approximate fluorescence response peak was selected in V1, three higher order visual areas, and a 'control' point outside of visually responsive cortex, and the pixels around this point in a 5 × 5 region were averaged to create ΔF/F traces. For each stimulus presentation, ΔF/F traces were segmented and baselined relative to the mean activity of the three frames prior to stimulus onset. Baselined ΔF/F traces were then further sorted by stimulus condition and behavioral state for comparative analyses. To calculate the mean locomotion speed during each trial, displacement of the mouse's position (as measured via an optical mouse placed tangentially to the spherical treadmill) was calculated for each trial. The units of displacement measured this way are arbitrary, and I picked a threshold for binarizing locomotion speeds into 'running' versus 'stationary' based on precedents set in our lab's previous work[8,109]. To calculate pupil diameter from IR face recordings, I used

DeepLabCut to track points on the perimeter of the pupil and a custom-built python script to calculate the longest diameter of the pupil from the tracked points. Mean pupil diameter was then calculated for each stimulus presentation. The units of pupil diameter measured this way are arbitrary, and a threshold for binarizing pupil diameter into categories of ‘large’ versus ‘small’ was picked based on the valley between peaks of the pupil diameter distribution across all animals and trials.

Statistical Analysis. In order to determine whether neural response thresholds were lower during behavior states of higher arousal, for each visual area a single sample t-test was performed to test for a statistical difference between the average neural threshold (C50) value for the high behavior state and the average neural threshold for the low behavior state. A Bonferroni correction was performed for multiple comparisons (one comparison for each visual area, corrected $p = 0.017$). This analysis was performed separately for trials separated according to locomotion state and pupil state.

CHAPTER 4: THE ROLE OF THE PULVINAR IN VISUAL ATTENTION

Abstract: The environment provides a vast amount of visual information, yet the brain has a limited capacity for information processing. Many studies of attentional control focus on local interactions that modulate cortical processing, and do not include the long-range effects of the thalamus on cortical visual dynamics. Yet, the pulvinar, a region of the visual thalamus, is in an ideal position to provide control over cortical information routing, as it has appropriate connectivity to relay information between cortical areas, and receives additional inputs that could regulate its function based on behavioral context. Yet, due to a paucity of genetic tools for manipulating specific cell types and pathways in primates, the precise role of the pulvinar in visual attention and cortical information routing remains unresolved. Here I challenge technical barriers to progress in studying pulvinar function by establishing a behavioral model of visual attention in mice, gaining access to more powerful genetic tools for pulvinar manipulation, and monitoring cortical information transfer via widefield calcium imaging. In this report, I describe the efficacy of chemogenetics (DREADDs: designer receptors exclusively activated by designer drugs) to suppress pulvinar activity, as well as performance on a visual selective attention task adapted from humans for mice. I find that mice can perform the adapted visual selective attention task, and perform similarly to humans in response to visual distractors. I also find that suppression of pulvinar using DREADDs did not impact cortical dynamics, and discuss the challenges of manipulating pulvinar activity with DREADDs using several different viral delivery methods.

Introduction.

The environment presents a vast amount of visual information, yet the brain has a limited capacity for information processing. Thus, a central challenge of visual processing is to selectively route behaviorally relevant information throughout visual networks, and limit distribution of irrelevant stimuli. For instance, while the driver of a car shares the same field of view as their passenger, different features of the environment need to occupy their attention. Although selective attention is a fundamental aspect of visual function, it is unclear how the brain selectively routes information throughout visual networks in order to achieve behavioral

goals. Many studies of attentional control have focused on local interactions that modulate cortical processing, but do not account for the long-range effects of the thalamus on cortical visual dynamics[110,111]. However, converging evidence suggests that thalamic regions, and particularly the pulvinar (LP), contribute to selective visual attention[112–115].

In both human and non-human primates, lesions of the pulvinar impair selective visual attention[116–120]. While pulvinar inactivation has no effect on performance in visual discrimination tasks, it increases the magnitude of the impairment in performance caused by visual distractors[116–120], indicating a role for the pulvinar in isolating behaviorally relevant stimuli from visual scenes. Indeed, neural responses in the pulvinar reflect the behavioral relevance of stimuli[112]. Recordings from primate visual cortex suggest that pulvinar may facilitate the preferential transfer of information about attentional priorities between areas by enabling synchronization[112,121–123]. Further, while visual processing is traditionally described as a hierarchy based on the direct transfer of information between cortical areas[124], cortico-cortical projection pathways are mirrored by an indirect pathway through the pulvinar[43–45,125–129], (**Fig. 4.1**). Importantly, the pulvinar also receives input from the prefrontal cortex and superior colliculus[46,115,127,130], situating it ideally to route visual information between cortical areas based on the integration of physical salience with behavioral context. If this is the case, inactivation of the pulvinar should result in broad changes in information flow at the level of cortical visual areas. Yet, little work has explored the effect of pulvinar manipulation on information routing simultaneously throughout the entire visual cortex.

Most studies of the influence of pulvinar on cortical processing have utilized electrophysiological recordings in primates, which restrict the size and number of cortical recording regions. And, due to a lack of genetic tools to manipulate specific cell types and pathways in higher species, it is not possible to cleanly manipulate pulvinar activity in primates[131]. While non-primate animal models provide greater genetic tools for neuronal manipulation, visual attention paradigms have been limited in this domain until recently[69,132–135]. Thus, the precise role of the pulvinar in selective visual attention, and the potential underlying circuit mechanisms, remain largely unknown. However, recent studies in mice have shown conclusively that mice perform selective visual attention in a manner similar to primates[69,132–135]. And, like the primate, rodent pulvinar has multiple subregions and receives input from primary visual cortex (V1), superior colliculus (SC), and extrastriate visual areas[45,127,136–138]. Thus, the

use of mice can overcome technical barriers to progress in studying pulvinar function in higher species by allowing access to cell-type specific tools for pulvinar manipulation, as well as population-level widefield calcium imaging across the entire visual cortex.

In order to use mice as a model organism to study the pulvinar's role in selective visual attention, I adapted a visual attention paradigm from human studies wherein pulvinar lesions impair task performance for use with mice, and used widefield calcium imaging of the visual cortex to test the efficacy of chemogenetics (DREADDs: designer receptors exclusively activated by designer drugs) to suppress pulvinar activity. I found that (1) mouse performance on the adapted visual task was similar to that of humans, (2) inhibitory DREADDs express weakly in LP, (3) suppression of LP using the DREADDs system did not impact cortical dynamics, and (4) a transgenic mouse line restricting expression of inhibitory DREADDs to calretinin-expressing neurons within pulvinar may selectively inhibit 'matrix' cells that, unlike the majority of pulvinar input to cortex, project sparsely and diffusely across the cortex, rather than in the targeted and topographic manner typical of thalamo-cortical projections.

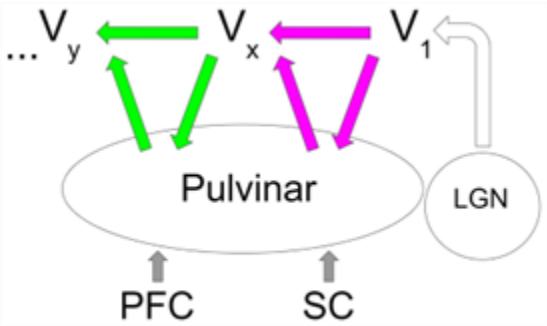


Figure 4.1. *Simplified schematic of visual pathways and thalamo-cortical connectivity.* While information transfer occurs directly between cortical visual areas, a secondary pathway also traverses the pulvinar.

Methods.

Visual Attention Task: I have developed a selective visual attention task for mice that is adapted from human pulvinar lesion studies. In humans, pulvinar lesions impair performance on a task in which participants must discriminate the orientation of a target stimulus when it is flanked by distracting luminance patches (**Fig. 4.2A**, top panel)[116]. Because this task relies on attentional mechanisms to filter out distractors and selectively process task-relevant information, it can be

used to test the role of the pulvinar in selective visual attention. Snow et al.[116] showed that the ratio of target and distractor contrasts determines task difficulty in control subjects and predicts the impact of pulvinar lesions on performance (**Fig. 4.2A,** bottom panel)). The adapted task is largely identical to that used by Snow et al. in humans[116]. Mice are required to ignore visual distractors to correctly discriminate the orientation of a target grating and receive a water reward. Distractors are luminance patches that horizontally flank the target, and task difficulty (i.e., demands on attentional control) can be manipulated by varying the relative contrast (i.e., relative physical salience) of target and distractor (**Fig. 4.2B**).

Behavioral Training: The behavioral training paradigm generally follows our lab's previously published approach[105]. In preparation for learning the task, mice are gradually habituated to head-fixation on a floating trackball. A stimulus display monitor is placed directly in front of the mouse, covering most of the mouse's visual field. Mice were trained to report the orientation of vertically or horizontally oriented square-wave gratings within a target patch by running right or left on a trackball. Performance was monitored and recorded during the task using custom MATLAB scripts.

Genetic Specificity of Chemogenetic Pulvinar Manipulation: While technical obstacles have prevented specific manipulation of the pulvinar in the past, new chemogenetic tools permit modulation of the activity of genetically defined neuronal populations. The DREADDs system allows for reversible inactivation of genetically defined populations of cells via injection of an otherwise inert ligand[139]. I used a transgenic mouse line that expresses Cre-recombinase in the calretinin-positive medial subregion of the pulvinar (**Fig. 4.3A**)[136], which projects to the dorsal visual cortex (**Fig. 4.3B**), providing genetic access to specific pulvinar subregions. I injected a Cre-dependent AAV virus (Addgene #44362) tagged with mCherry into the pulvinar of these mice to selectively express the inhibitory DREADD receptor hM4Di. Control mice were injected with virus that expresses mCherry alone (Addgene #50459). In addition to the transgenic mouse line that expresses Cre-recombinase in the calretinin-positive medial subregion of the pulvinar, I also used gCaMP6s mice combined with several other methods of delivering DREADDs to LP, including non-cre-dependent inhibitory DREADDs, cre-dependent inhibitory DREADDs delivered to LP simultaneous with a retrograde virus injected in V1 expressing cre-recombinase

(resulting in LP neurons that project to V1 will express Cre), as well as genetic ablation of LP with a caspase-expressing virus.

A B

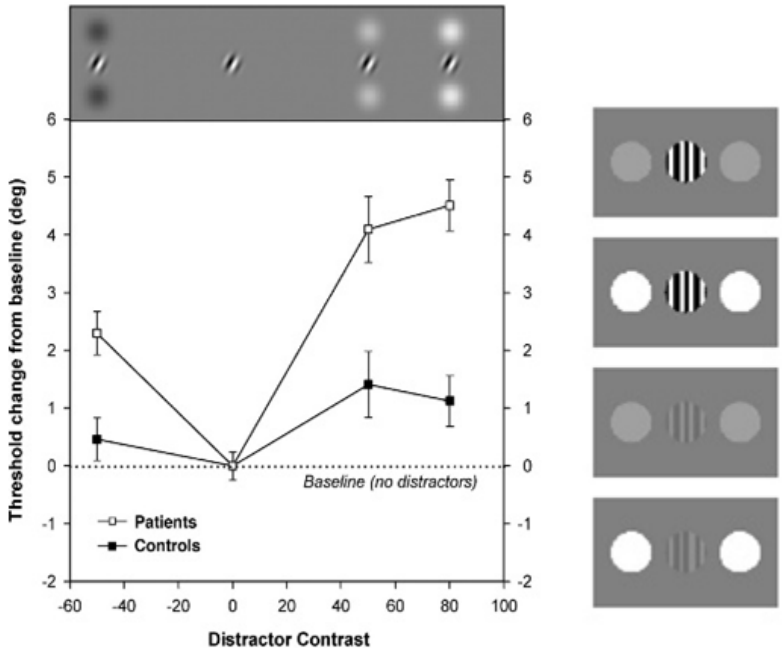


Figure 4.2. *A Selective Visual Attention Task Adapted from Humans for Mice.* (A) From Snow et al., 2009. In a visual attention task, participants discriminate the orientation of a target stimulus flanked by distracting luminance patches (top). In humans with pulvinar lesions, the contrast of the distractor has greater impact on the detection of the target (bottom). (B) The stimuli in the adapted task are largely identical to Snow et. al, 2009, adapted for mice.

Transgenic Mouse Line: In order to perform functional imaging across wide regions of cortex, our lab has generated a transgenic mouse that expresses the ultra-sensitive calcium indicator GCaMP6s under the control of the tetO promoter. By crossing this line to a CaMK2-ttA driver line, I expressed GCaMP6s in excitatory pyramidal neurons throughout the cortex. I crossed these GcaMP6s expressing mice with the Calb2-Cre mouse line which expresses Cre-recombinase in the calretinin-positive medial subregion of the pulvinar (**Fig. 4.3**).

DREADD Delivery: The inhibitory DREADD receptor hM4Di was delivered to the pulvinar via an AAV viral injection that expresses hM4Di tagged with mCherry. The viral delivery of an mCherry vector without hM4Di was used as a control. Mice were also implanted with a titanium headplate used for stabilization during head-fixed behavior. Viruses were injected in quantities

ranging from 40-100nL (with earlier experiments receiving less and later experiments receiving more).

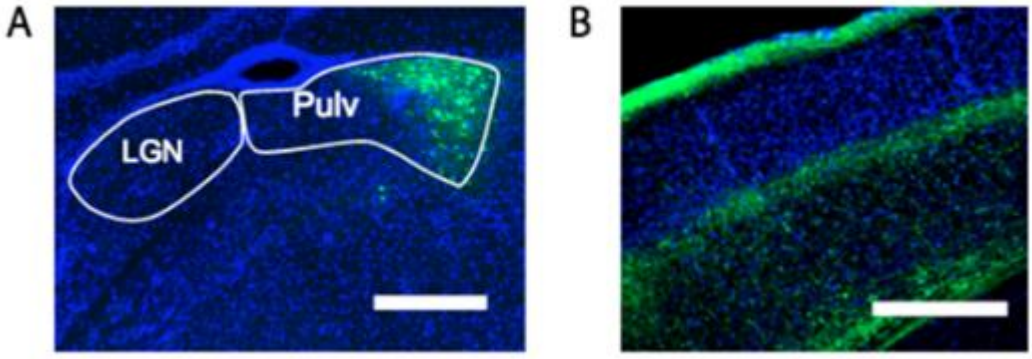


Figure 4.3. *Genetically-Specific Access to Pulvinar Sub-region.* (A) Injection of Cre-dependent GFP into a transgenic mouse line expressing Cre-recombinase in the calretinin-positive medial subregion of the pulvinar, which projects to the dorsal visual cortex, shown in (B).

Surgical Preparation: I have optimized surgical techniques to implant a coverslip secured to the surface of the mouse skull with cyanoacrylate for chronic calcium imaging. With this preparation were able to record summed activity of superficial cortical neurons (layers 1-3) spanning the dorsal cortex with widefield imaging. Mice were implanted with coverslips during the same surgical session used for DREADD delivery.

Widefield Calcium Imaging: Widefield imaging allowed for the simultaneous monitoring of cortical dynamics over nearly the entire dorsal surface of cortex, including V1 and extrastriate areas. Using a custom-built macroscope with a tandem lens design, I was able to image the cortical surface with 50 um and 10 Hz resolution, including the sequential activation of cortical areas (**Fig. 4.4A**) during the selective attention task, which I then mapped onto visual areas based on functionally-defined retinotopic maps using topographical stimuli and computational methods described previously in Wekselblatt & Niell et al.[22] (**Fig. 4.4B**).

Visual Stimuli During Passive Viewing: Passive viewing stimuli consisted of grated patches presented at various orientations, spatial frequencies, and locations within the visual field, and were presented at the right eye only (while recording occurred in the left visual cortical hemisphere).

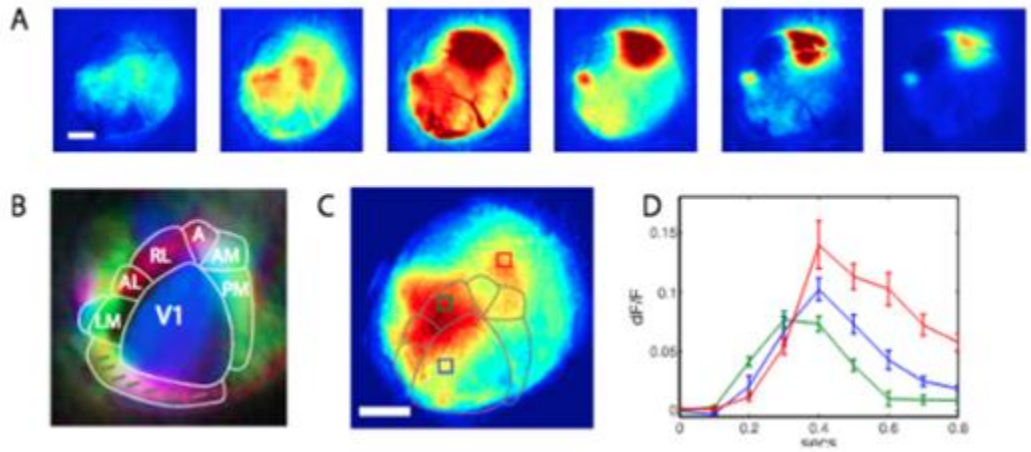


Figure 4.4. *Monitoring of Cortical Dynamics.* (A) Changes in large-scale activation patterns across the dorsal cortex during passive viewing. (B) Functionally defined retinotopic maps. (C) Spatial activation map, with potential regions of interests highlighted. (D) Temporal activation profiles of cortical visual areas. Adapted from Wekselblatt et. al, 2016.

Chemogenetic Inactivation: Clozapine was injected in DREADDs mice in order to suppress pulvinar activity during passive viewing. Widefield calcium imaging was performed for the duration of passive viewing (30-60 minutes), and for the duration of the retinotopic stimuli used to functionally define visual areas. To avoid confounds of non-specific effects of clozapine[140]. comparisons were performed between DREADD+mCherry and mCherry-only controls, with both groups receiving clozapine. Based on existing literature, clozapine was administered in dosages ranging from 0.1-2.5 mg/kg, above which off-target motor effects were observed. Most mice received a dose in the range of 0.5-1.0 mg/kg. In all experiments, the wait time between clozapine injection and imaging was around 20 minutes.

Results.

Training and Performance on Selective Visual Attention Task ('Flanker Task'). Mice are able to learn the orientation discrimination task without distractors at 80-90% accuracy after 2-4 weeks, after which distractors are added to each trial. Mice can perform hundreds of trials during each experimental session. The results show the same dependence on contrast in human controls is true in mice - for a given target contrast, performance decreases as distractor contrast increases (**Fig. 4.5**). While lesion studies in humans show an association between pulvinar activity and

attentional control, the adaptation of this task for mice enables future experiment to precisely inactivate a specific subregion and probe the neural dynamics that underlie this control.

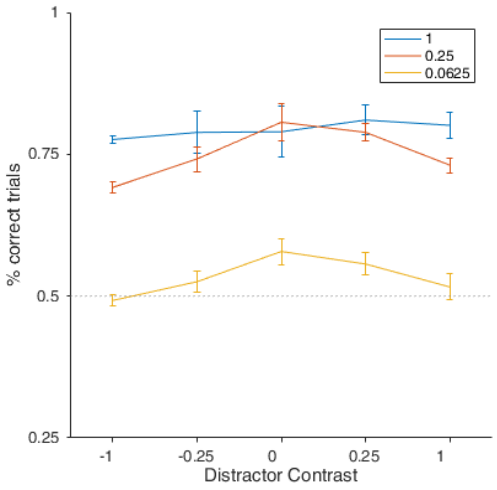


Figure 4.5. *Performance on Visual Selective Attention Task for One Example Mouse.* As with humans, mouse performance decreases as distractor contrast increases. A distractor contrast of +1 is at full contrast and white, whereas a distractor contrast of -1 is at full contrast and black. The three colored series represent target contrasts (100%, 25%, and 6.25% contrast).

Identifying LP and LGN Coordinates for Viral Injection Sites. To narrow in on the viral injection sites, bilateral DiO injections were performed in both LGN (**Fig. 4.6**, top) and LP (**Fig. 4.6**, bottom). Injection coordinates were based on where the structure in question had the greatest area (as determined by both the DiO injections and by the Allen Mouse brain atlas).

LGN: Non-Cre-Dependant Inhibitory DREADDs Expression and Efficacy. Once the injection site coordinates were identified, non-cre-dependant iDREADDs injections into the LGN of mice expressing gCaMP6s in cortex were performed as an intended positive control to show that the DREADDs system worked to suppress activity in the LGN (indicated by a decrease in input to V1 as measured with widefield calcium imaging). However, getting the virus to express strongly within LGN proved difficult (**Fig. 4.7**), and there were no changes in calcium activity observed after clozapine administration in any of the LGN mice.

Since DREADDs suppression of LGN was unsuccessful and not critical to the study, that goal was abandoned for the sake of time. Still, the calb2-cre mice selectively expressing iDREADDs

in LP were not yet born, and as a result the next experiments performed consisted of non-cre-dependent iDREADDs injected into the LP of gCaMP6s mice.

Note that, in early experiments, 40-50nL of virus were injected, but over time that grew to 100 nL. Also, initially all injections were performed bilaterally, but later on injections were performed unilaterally. Whether the injections were bi-or uni-lateral had no impact on pre-post changes in fluorescence for any mice, under any conditions.

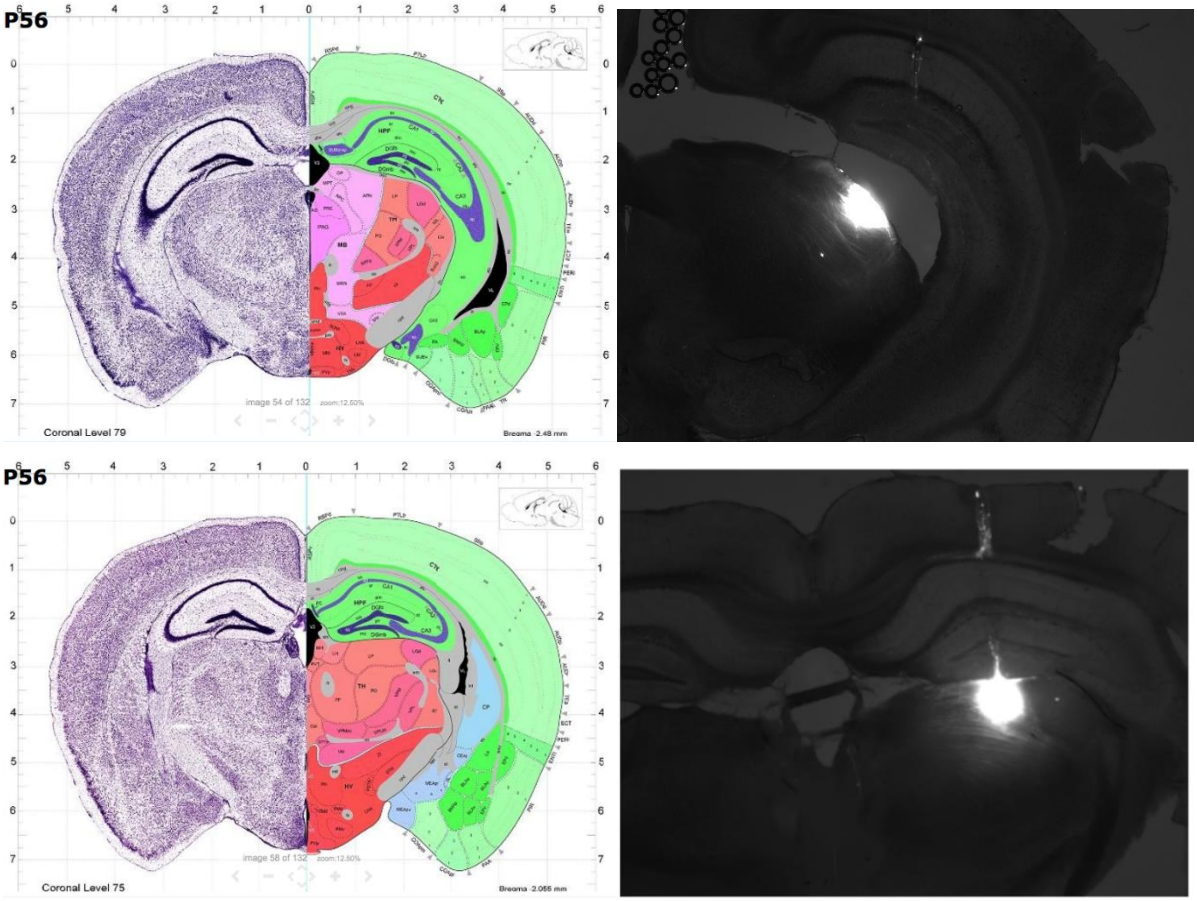


Figure 4.6. *Injection Sites*. Top: (left) LGN injection site identified in Allen Mouse brain atlas (from bregma: -2.45 A-P, +/- 2.3 M-L, from brain (bevel deep): -3.2 D-V.) and in brain tissue using DiO injection (right). Bottom: (left) LP injection site identified in Allen Mouse brain atlas (from bregma: -2.0 A-P, -1.5 M-L, from brain (bevel deep): -2.5 D-V) and in brain tissue using DiO injection (right).

LP: Non-Cre-Dependant Inhibitory DREADDs Expression and Efficacy. Despite relatively robust expression of non-Cre-dependant iDREADDs in LP (**Fig. 4.8**) revealed upon sectioning, no pre-post changes in widefield signal were observed in any of these mice (**Fig. 4.9**).

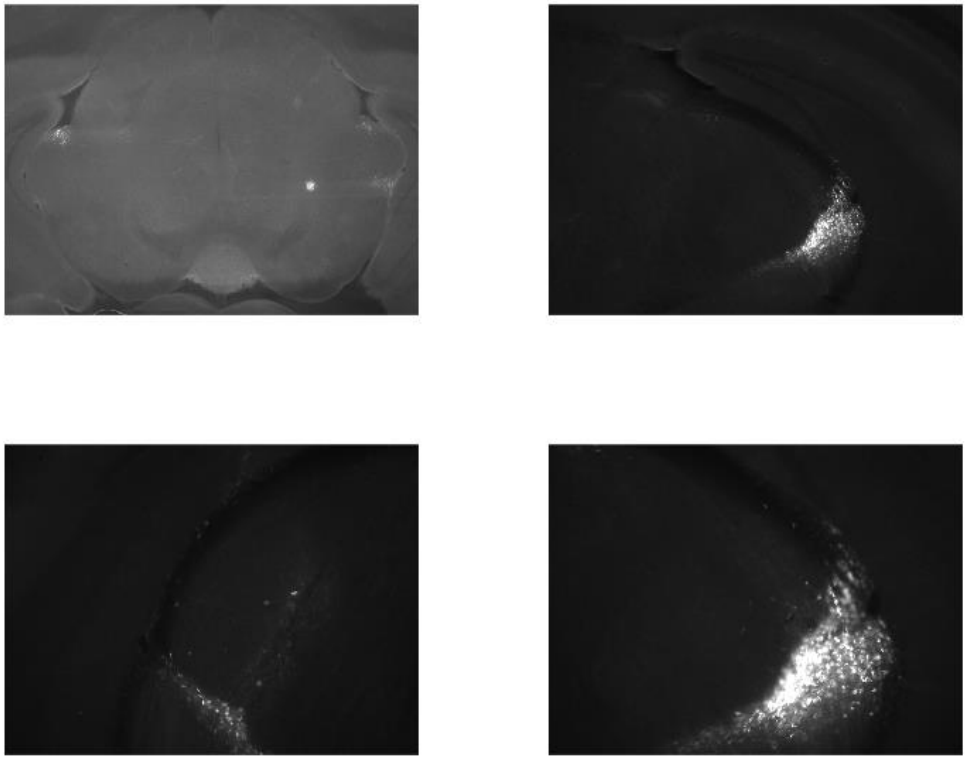


Figure 4.7. *Weak Expression in LGN for non-cre-dependent inhibitory DREADDs*. Results from typical bilateral non-cre-dependent iDREADDs injection into LGN. Panels move posterior to anterior in clockwise direction.

LP: Cre-Dependant Inhibitory DREADDs Expression and Efficacy. Cre-dependant iDREADDs were expressed within LP by two different methods. *(1) Retrograde cav2-cre in V1 + cre-dependent iDREADDs in LP.* Still waiting for the calb2-g6 mice to be produced, I performed 'intersectional' injections with cav2-cre (retrograde) in V1 and cre-dependent iDREADDs in LP. The goal here was to isolate a portion of LP neurons that project to V1. Sectioning of these mice showed light expression and I observed no effect of cortical activity on widefield signal post clozapine administration (**Fig. 4.10**).

(2) Cre-dependent iDREADDs in LP of calb2cre-ck2g6 Mice. Sectioning of these mice showed moderate iDREADDs expression which did appear limited to the boundaries of LP (**Fig. 4.11A**). I observed no effect of clozapine administration on cortical activity (**Fig. 4.11B**).

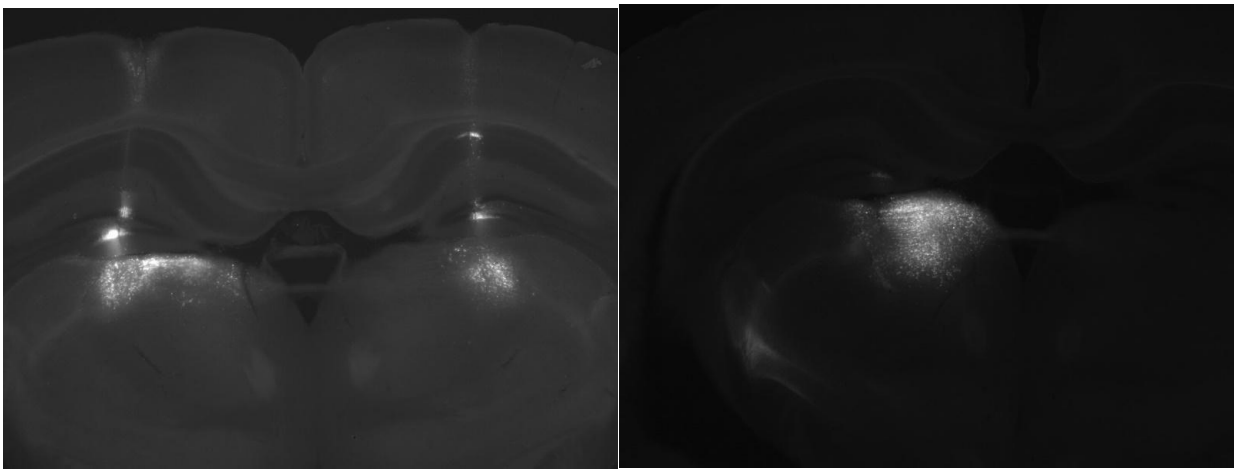
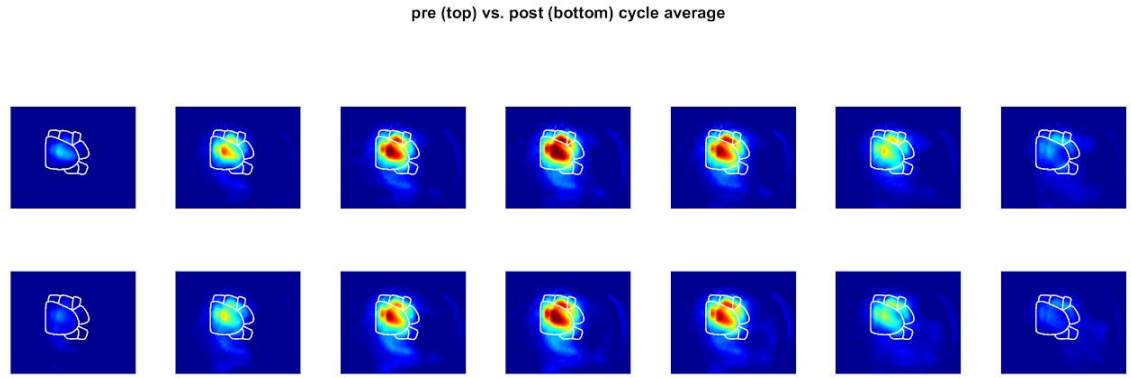


Figure 4.8: *Expression patterns of injections of non-cre-dependent iDREADDs into LP of two example gCaMP6s mice.* Left: bilateral injections. Right: unilateral injections.

Genetic Ablation of LP of calb2cre-ck2g6 Mice. I then took a more blunt approach and performed some cre-dependent caspase3 injections (along with non-cre tdTomato for visualization of cell death) in order to genetically lesion LP in calb2cre-ck2g6 mice. Since no injection of clozapine was given during these experiments (no iDREADDs), I imaged the mice at week 1 and week 3 post viral injection, to compare pre and post lesioning. I was successfully able to lesion portions of LP this way (**Fig. 4.12A**), and calcium imaging showed an increase in the amplitude of cortical peri-stimulus response post lesioning in these mice (**Fig. 4.12B**).

In conclusion, only calb2cre-gCaMP6s mice with genetic lesions from Cre-dependant caspase3 showed any pre-post changes in dorsal visual cortical calcium activity. There were no changes observed in visual cortical activity for gCaMP6s mice with non cre-dependant DREADDs in LGN or LP, nor for gCaMP6s mice with cre-dependent DREADDs in LP via intersectional method with cav2cre in V1, nor for calb2cre-gCaMP6s mice with cre-dependant DREADDs in LP.

A



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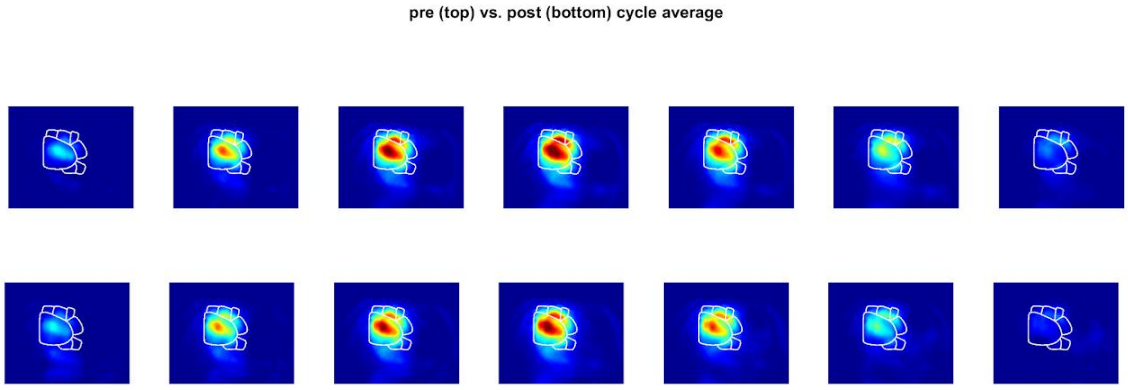


Figure 4.9. *Calcium Imaging During Passive Viewing.* (A) Peri-stimulus response pre (top) and post (bottom) Clozapine in one example control mouse. (B) Peri-stimulus response pre (top) and post (bottom) Clozapine in one example mouse with non-cre-dependant iDREADDs in LP.

Discussion.

The most notable result obtained was an increase in cortical activity across the entire visual cortex post lesioning with Cre-dependent caspase3 (n=1). This result could suggest that the input from LP to cortex is mainly inhibitory. However, the increase in cortical activity observed post lesioning could also be due to compensatory changes in cortical activity resulting from the loss of excitatory input from pulvinar. Considering that this heavy-handed method of pulvinar suppression is not conducive to experiments where mice have to learn and perform a visual behavior task over time, a mechanistic understanding of the increase in cortical activity post caspase lesioning was not pursued.

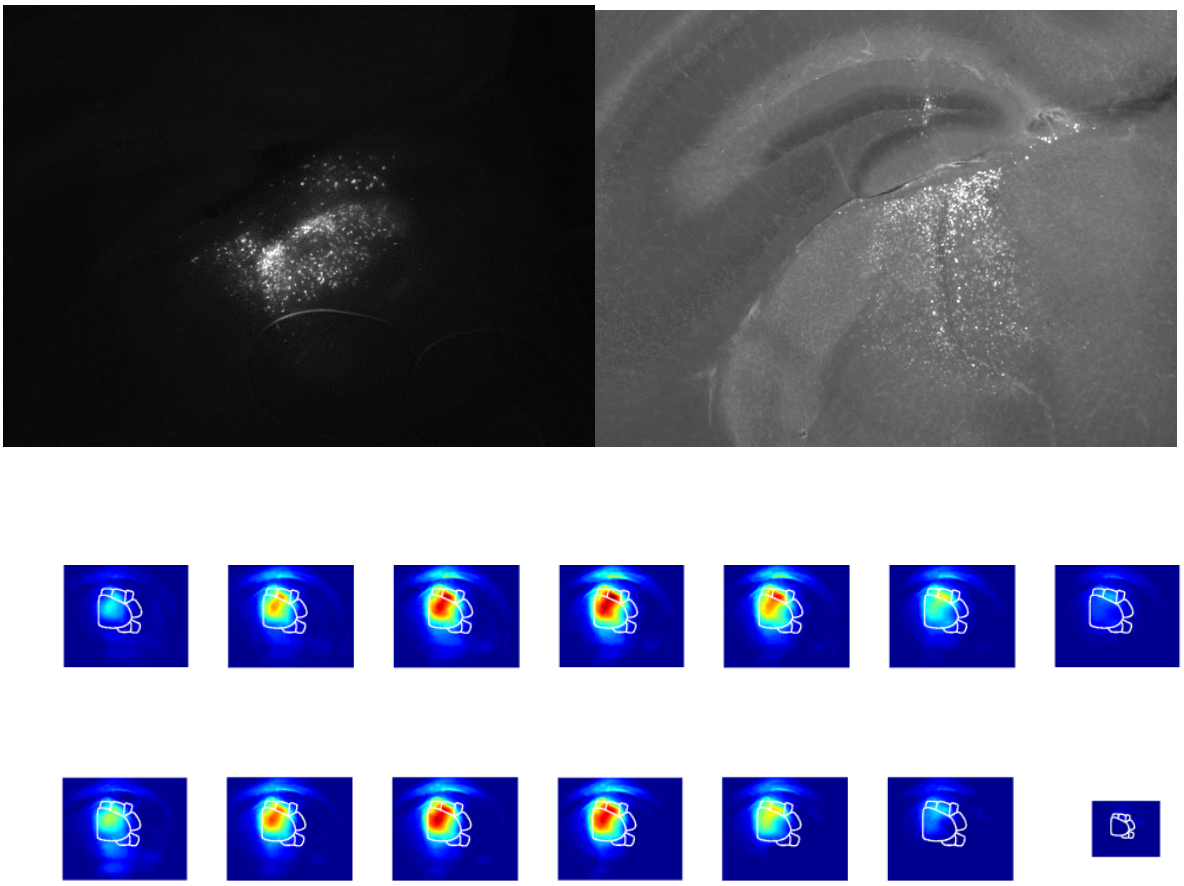
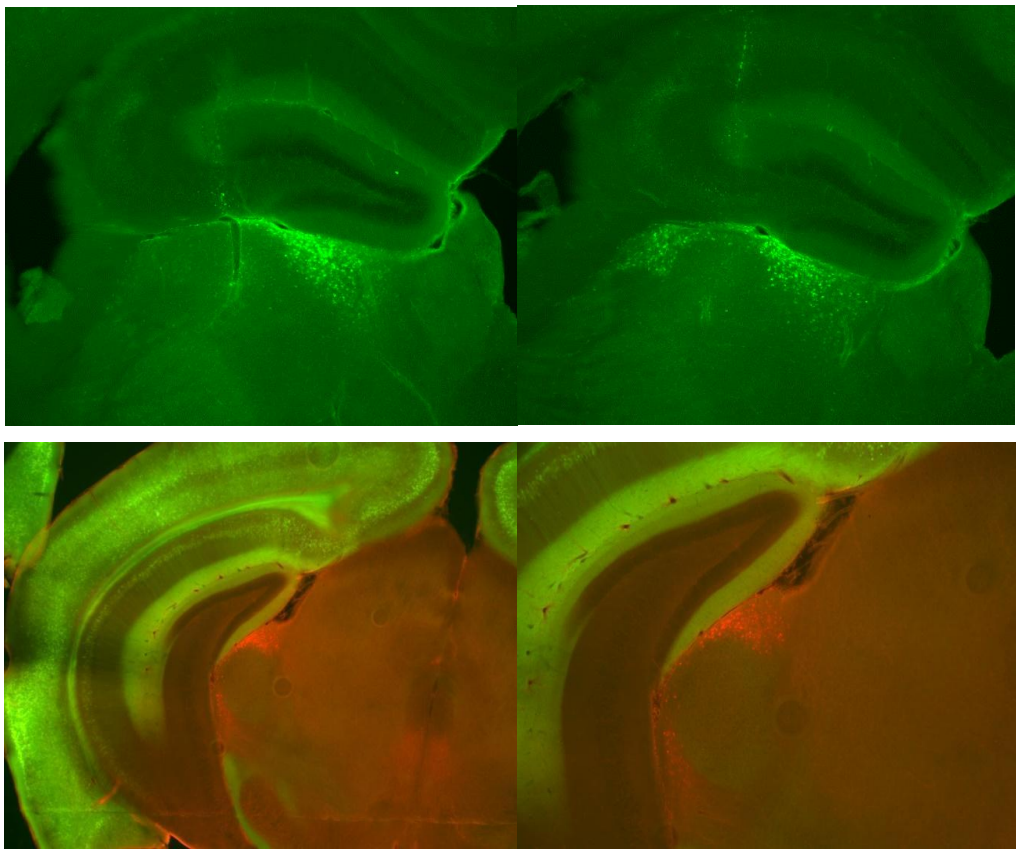


Figure 4.10. *Effect of Intersectional Method (using Retrograde cav2-cre in V1 to express cre-dependent iDREADD in LP).* Top: Expression of cre-dependent iDREADD in LP using Retrograde cav2-cre in V1 in two example mice (left and right). Bottom: Peristimulus neural activity pre (top) and post (bottom) clozapine administration for one example mouse.

While this report demonstrates problems with weak cellular expression and undetectable LP suppression (as measured indirectly by cortical monitoring) across all methods of DREADDs delivery, there is evidence that the transgenic line of mice selectively expressing cre-recombinase in calretinin expressing LP neurons may be an especially poor candidate for suppression of LP input to cortex. By cross referencing my own sectioning images with those from the Allen Institute's mouse atlas for the Calb2-IRES-Cre transgenic mouse line, as well as previously published work using this mouse line[136] and describing projections patterns from LP to cortex[45], I concluded that this transgenic line may label diffuse 'matrix' cells in LP rather than 'core' cells that project topographically discrete input to cortex. If this is the case, DREADDs suppression of these calretinin expressing neurons in LP may not induce changes in cortical

A



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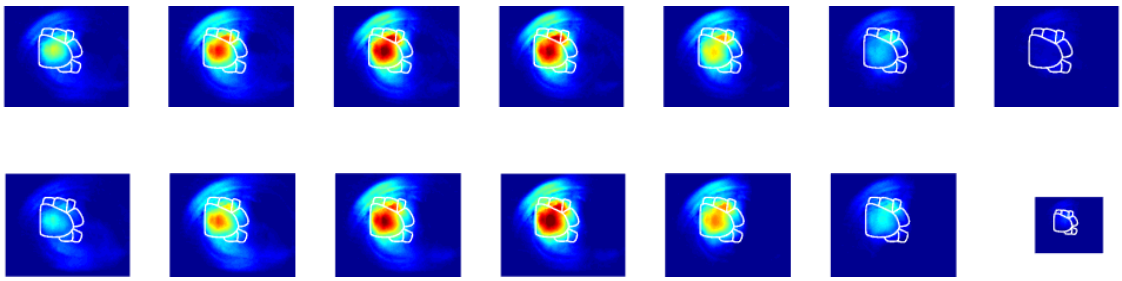
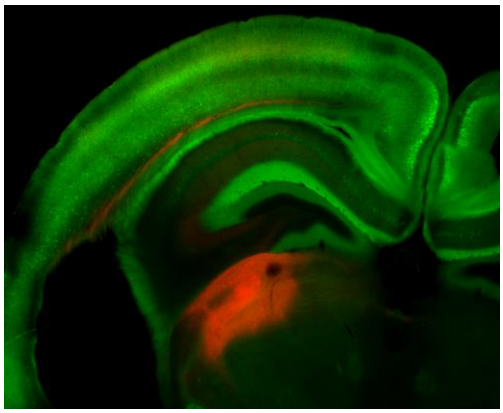


Figure 4.11. *Effect of Cre-dependent iDREADDs in LP.* (A) Expression of cre-dependent iDREADD in LP in two example calb2cre-ck2g6 mice (top and bottom rows). (B) Peristimulus neural activity pre (top) and post (bottom) clozapine administration for one example calb2cre-ck2g6 mouse.

A



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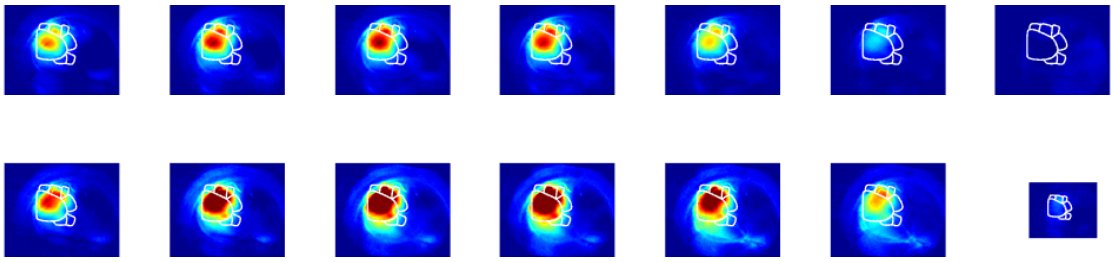

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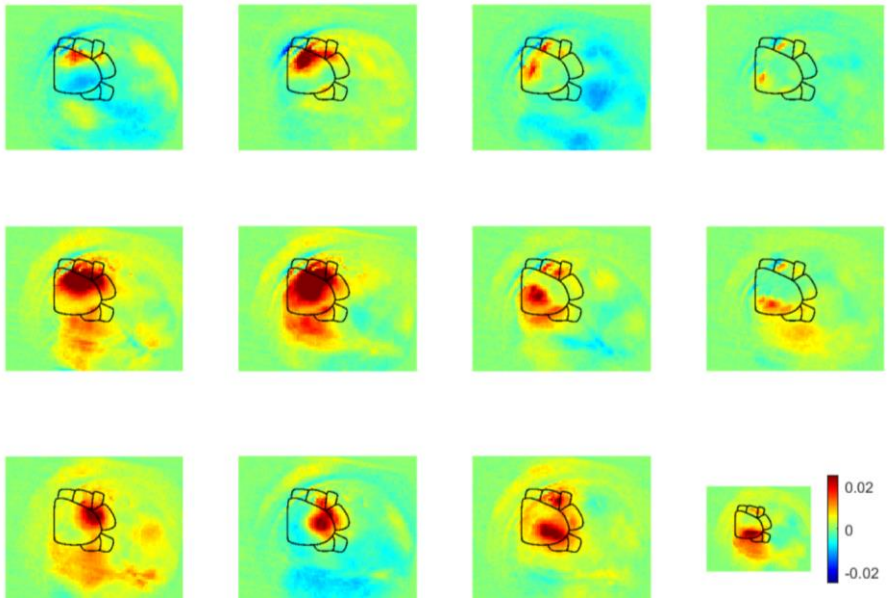

Figure 4.12. *Genetic lesioning of LP using caspase3 in calb2cre-ck2g6 mice*. (A) Sectioning showing caspase induced cell death in LP for one example calb2cre-ck2g6 mouse. (B) Peristimulus activity pre (top) and post(bottom) lesioning in one example mouse. (C) Post Response Minus Pre Response per location on monitor of each stimulus for one example calb2cre-ck2g6 mouse.

processing that are observable via widefield calcium imaging of cortex, because firstly, matrix neurons project sparsely and diffusely to cortex[45,141–143], and secondly, matrix neurons are hypothesized to be involved in signaling behavioral context, and the present study's imaging sessions took place during passive viewing[45,141–143]. There is also evidence to suggest that certain regions of LP may be most sensitive to movement, rather than stationary stimuli[127].

Further support for the hypothesis that the calretinin expressing neurons in LP are matrix cells was observed when I compared retinotopic mapping patterns in calb2-cre mice where gCaMP was expressed Cre-dependently in calretinin-expressing LP neurons versus wildtype mice expressing Cre-dependent gCaMP in LP via a paired retrograde expression of Cre-recombinase (using the cav2-cre virus in V1, which receives projections from LP) (**Fig. 4.13**). However, while Cre-dependent gCaMP expressed via calb-cre neurons in LP showed much sparser retinotopic activity than Cre-dependent gCaMP expressed via retrograde cav2-cre virus injected into V1, this could be a reflection of the fact that calb2-cre neurons in LP are less sensitive to retinotopic stimuli than LP neurons that project to V1, so the interpretation of this result is unclear.

Another big challenge during this project was the persistent appearance of abnormal holes in animal brains observed upon sectioning of mice expressing iDREADDs (**Fig. 4.14**). These abnormal holes in brain tissue occurred about 50% of the time. The phenomena of finding holes in the sectioning results occurred as early as the first group of mice that received virally-delivered DREADDs injections, and continued to be observed though the entire ~3 years that this study was ongoing. Extensive steps were taken to try to identify the cause of these holes, including painstaking efforts to make sure there were no air bubbles in the syringe during viral delivery of DREADDs, no unintentional tearing of brain tissue during the injections themselves, varying the virus titer and serotype of the virally-delivered DREADDs, and varying the time period between injection of DREADDs and sectioning of the brain tissue (a range of 3-8 weeks, note that the virus takes 2-3 weeks to express). The holes were observed 50% of the time regardless of the specific virus type (i.e., cre-dependent, non-cre-dependent, intersectional-cre, etc). The holes were also observed at a rate of 50% regardless of the lab member (4 total - KC, PP, EL, IE) who performed the viral injections. None of the mice with holes in their brain tissue showed pre-post changes in WF signal. Unfortunately, this problem was never solved, and there

are no clear suggestions regarding how to address this matter in the future for similar experiments the lab may perform.

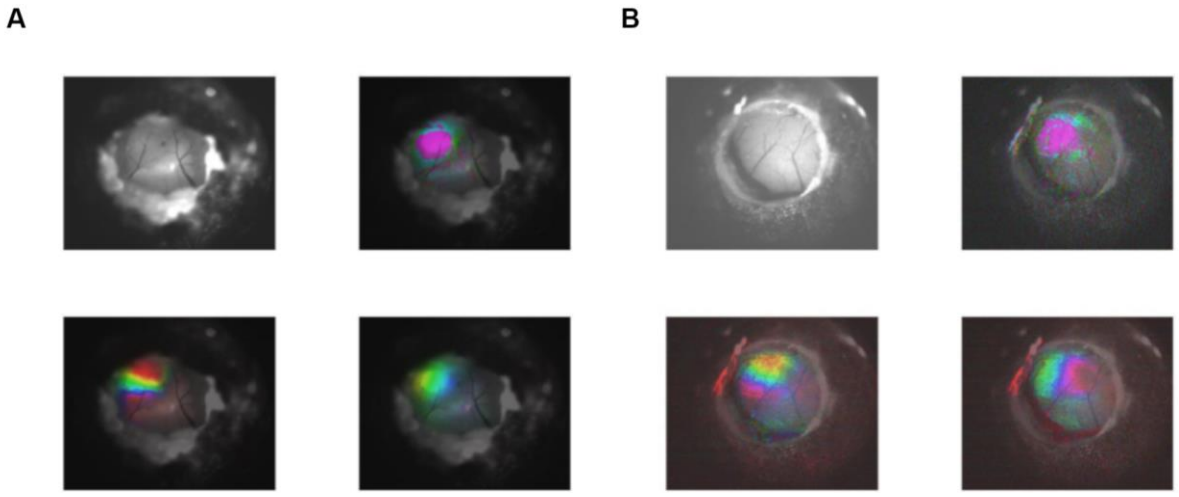

Figure 4.13. *Sparse Retinotopy of Calb-Cre Neurons.* (A) Retintopic activity in neurons expressing gCaMP via intersectional viral delivery of Cav2-Cre in V1 and Cre-dependant gCaMP in LP. (B) Retintopic activity in neurons expressing gCaMP via Cre-dependent gCaMP in LP of Calb2-cre mice.

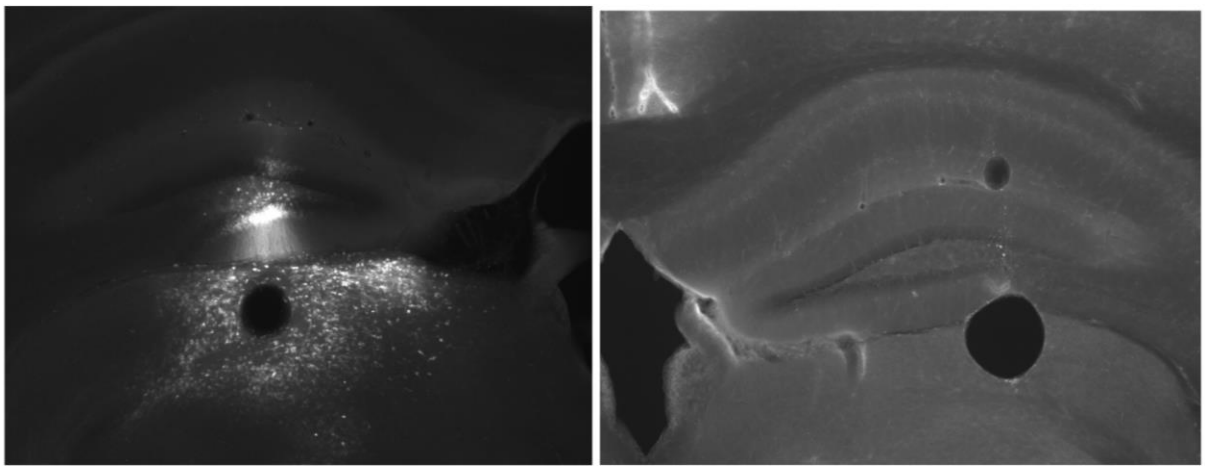

Figure 4.14. *Abnormal Holes in Brain Tissue.* Example abnormal holes observed in two example mice (left and right). Sectioning performed ~3 weeks post viral injection with iDREADDs.

Future Directions.

Going forward, there are two main challenges if DREADDs are to be used to test the role of LP in selective visual attention and cortical information processing. The first challenge is to understand and remove the source of the abnormal holes observed in brain tissue after viral delivery. This is a serious problem spanning all virus types and lab personnel. The second challenge will be to determine whether the calb2-cre line of mice is appropriate for testing the role of a genetically-restricted subset of LP neurons in selective visual attention and cortical processing. If the calb2-cre line were to be continued to be used for this purpose, it would be advisable to explore using a different method of cortical monitoring, perhaps 2-photon calcium imaging at the cell ensemble level, rather than widefield calcium imaging at the population level. It would also be advisable to test a wider range of visual stimuli during passive viewing and LP suppression, such as movement stimuli, which matrix neurons and/or certain portions of LP may be more responsive to. Importantly, it may be the case that suppression of calretinin expressing neurons in LP will only show changes in cortical activity if mice are actively engaged in the adapted visual attention task. If the calb2-cre line of mice are to be abandoned for this purpose, then either another genetically specific population of LP neurons would need to be identified for manipulation, or another method of DREADDs delivery should be used, such as an intersectional method for cre-dependent DREADDs expression in LP, or simply using non-cre-dependent DREADDs in LP. If non-cre-dependent DREADDs are to be used, care must be given to localize the expression of DREADDs to LP only, and exclude surrounding areas such as LGN.

If this investigation were to be continued in the future by the lab, perhaps the most sensible first step would be to perform electrolytic lesions of LP and determine whether changes are observed post lesioning with whatever method of brain activity monitoring is used (2-photon, widefield, etc). Electrolytic lesions occur over a much shorter time frame than genetic lesions (which need time for virus to express), so this method could help exclude the possibility that changes in cortical processing post-lesions are compensatory rather than indicative of LP's role in cortical processing. If electrolytic lesioning of LP does not result in changes to cortical processing, it is unlikely that DREADDs suppression of LP will show changes to cortical processing either. It may also be advisable to confirm effective suppression of pulvinar activity

by recording from the pulvinar using silicon probes or by performing two-photon imaging of pulvinar axons projecting to V1.

If these two challenges (abnormal holes, specificity of pulvinar manipulation) were overcome, it would be possible to perform an experiment where chemogenetics are used to suppress neural activity in LP during the adapted visual attention task and concurrent cortical monitoring. Comparisons of performance as a function of stimulus parameters with and without pulvinar suppression would indicate whether the LP is required to maintain visual selective attention. In addition to detecting large-scale spatial and temporal changes in visual responses, it may then be possible to apply machine learning algorithms to calcium imaging data, decode stimulus representations, and detect changes to information routing within visual cortex. Comparisons of behaviorally relevant stimulus representation in extrastriate areas with and without pulvinar suppression would indicate whether the pulvinar selectively routes visual information. This experimental paradigm would provide detailed measures of both behavioral performance and the dynamics of neural activity, which would facilitate pinpointing the specific role of pulvinar in selective visual processing. For example, with the adapted behavior task I can dissociate effects on baseline performance from performance with distractors, as well as varying relative contrast of the target and distractors, as in Snow et al[116]. Likewise, the use of widefield calcium imaging allows the determination of not only whether pulvinar input increases or decreases cortical activity, but also how the flow of information across areas is impacted. Completion of these aims would help determine the role of the pulvinar in selective visual attention and cortical visual processing.

Defining the role of thalamocortical circuits in vision is a key step towards developing a full account of sensory processing, and ultimately, the functional logic of brain-wide connectivity. The information contained within this report, as well as the suggested future studies, will help demonstrate the role of the pulvinar in visual selective attention and information processing across cortical visual areas, furthering our understanding of cortical and subcortical interactions in visual processing.

CHAPTER 5: CONCLUSION

Collectively, the work presented in this dissertation constitutes an exploration of the neural correlates of consciousness using mice as a model organism and vision as a model modality. In review, chapter 2 discussed the translation of human consciousness research to mice and vice versa, and found that a good deal of consciousness research involving mice has already begun. Chapter 3 determined the impact of behavior state on cortical responses to weak visual stimuli, finding that locomotion and arousal decrease neural thresholds for contrast. Chapter 4 explored the role of the visual thalamus in directing selective visual attention, finding that mice can learn and perform similarly to humans on an adapted visual selective attention task, and discussing the challenges of manipulating pulvinar activity with chemogenetics (DREADDs) using several different viral delivery methods.

In the introduction of this dissertation, three motivating questions were put forth: (1) Can the use of mice drive forward theories of consciousness developed in primates? (2) Does behavior state impact the neural response to near-threshold visual stimuli in mice? (3) Where does conscious awareness enter along the visual processing hierarchy?

(1) Mouse as a model organism for studying consciousness: The literature reviewed in Chapter 2 demonstrates that mice are an appropriate model organism for the study of consciousness because they can perform many of the canonical visual tasks used in human studies of consciousness, have many shared features of brain organization (including hierarchical stimulus information processing), and, due to advances in genetic tools for neuronal recording and manipulation, are preferable to primates for the investigation of causal mechanisms underlying conscious perception. Excitingly, there is already some pioneering usage of fMRI[93] and EEG[94] emerging in mouse studies. Recently, there has been increasing use of lightweight eye tracking options[62,144] for recording eye movements in mice under freely moving conditions, which is ideal because head-fixation limits eye movements (particularly in mice, who rely heavily on the angle of their head to direct their line of sight).

In chapter 3, an advantage of using mice to study visual consciousness is demonstrated - the use of mice allowed the testing of the impact of behavior state on neural thresholds by monitoring cortical visual activity at spatial and temporal resolutions higher than fMRI (via the

use of GCaMP6s mice and widefield calcium imaging) while importantly, the mouse moved "freely" on a low-friction trackball. Monitoring of neural activity in visual regions during uninstructed movement is not easily performed in primates because it is difficult to have a head-fixed recording set up that allows for locomotion (relatedly, it is uncertain whether movement has as large of an impact on visual processing in primates as it does in mice[145,146]). And, the use of mesoscale imaging in mice allowed for the comparison of neural responses to a single stimulus across all cortical visual areas simultaneously.

Chapter 4 discusses experiments where mice allowed for the usage of chemogenetics (DREADDs: designer receptors exclusively activated by designer drugs) as well as genetic ablation to suppress pulvinar activity. Chapter 4 also showed that mice can perform a visual selective attention task adapted from humans for mice, and that, as with humans, for a given target contrast, performance decreases as distractor contrast increases.

(2) Impact of behavior state on neural thresholds: The results of experiments performed in chapter 3 suggest that an animal's behavior state alters neural sensitivity to weak visual stimuli, helping to explain why stimuli impacting the retina are only sometimes consciously perceived, and other times go unseen. Experimental results from chapter 3 demonstrate that states of greater arousal (such as during running or pupil dilation) increase the contrast sensitivity of cortical regions. This observation is congruent with the ethologically based hypothesis that behavior state modulates neural response gain in order to improve the detectability of weak stimuli under natural conditions such as at dusk or dawn, and the mechanistically-based hypothesis that motor signals that depend on behavior state factors such as pupil dilation and locomotion increase neural response gain, thereby enhancing stimulus processing during states of higher arousal and movement[9–11]. Such a process could potentially modulate the visual threshold based on behavioral context, enhancing detection of weak stimuli when they are behaviorally relevant and/or temporally coincide with peaks in periodic oscillations in baseline arousal.

While most existing studies of behavioral state impacts on cortical visual processing use salient stimuli (such as full-field stimuli) or do not continuously vary detectability near the threshold of visual detection, the experiments presented in chapter 3 did, thus comprising a unique contribution to the study of the effect of behavioral state on neural responses to near-threshold stimuli. Interestingly, results from experiments performed in chapter 3 show that, at the

population level in visual cortex, visual stimuli as brief as 100 ms elicit a dynamic range of neural responses when presented across a range of stimulus contrasts, which is consistent with a mouse study by You and Mysore[77] discussed in chapter 2's literature review which demonstrated that mice can report targets as brief as 100 ms. While estimates of perceptual thresholds for contrast in mice vary widely depending on the method of measurement used, a study by Histed et al.[75] varied the contrast of full field gratings at a single orientation in a absolute detection task and found that mice could detect the gratings when contrast was as low as 2% (with spatial frequency of 0.1 cpd). In chapter 3's experiments, I found neural thresholds to be between 12-50% (with spatial frequency of 0.16 cpd) depending on behavioral state and visual area, suggesting that, at the very least, my measured neural thresholds for contrast are not lower than any reported perceptual thresholds for the contrast of similar stimuli. Another study discussed in chapter 2[2] demonstrated that pupil diameter is predictive of performance on visual discrimination tasks, suggesting that the impact of pupil diameter state on neural thresholds observed in chapter 3's experimental results may truly correspond to changes in perception.

(3) Theories of Consciousness: The mouse literature discussed in chapter 2 as well as the experiments performed in chapters 3 and 4 shed light on how the use of mice can help determine which brain regions are involved in generating conscious awareness. For instance, a study by Glickfield et al.[78] provides evidence that V1 plays a role in determining the threshold of detection in mice. By optogenetically suppressing V1 during a contrast and orientation change detection task, they showed that suppression of V1 increases the threshold for change detection (although, even when V1 was maximally suppressed, visual perception was not eliminated entirely). Further evidence in mice for the role of early visual cortex in generating consciousness is presented by Gale et al.[79] in another study discussed in chapter 2. Gale et al. used optogenetics in a backward visual masking task to silence mask-evoked activity and restore performance to no-mask levels. The results of their study suggest that stimulus processing in V1 can determine whether or not a mask inhibits perception of a target stimulus that comes before it. Another study by Glickfield et al.[80] investigated the role of higher order visual areas in supporting visual perception in mice. They showed that suppression of V1 and lateral higher order visual areas (LM, AL) decreased performance by increasing perceptual thresholds on a contrast and orientation detection task. In contrast, suppressing PM (which is higher than LM and AL in the

visual processing hierarchy) selectively increased false alarm rates, suggesting that primary and lateral visual areas may be responsible for the perception of orientation and contrast, whereas more medial and anterior regions may play a role in decision-making instead. These results in mice are especially consistent with the "Posterior Hot Zone" theory of consciousness and the related Integrated Information Theory of Consciousness. However, a role for primary and lateral visual cortex in generating conscious perception is not strictly inconsistent with recurrent processing or global neuronal workspace theories of consciousness. This is because, amongst other reasons, none of these studies have ruled out a concurrent role for frontal parietal networks or top-down feedback control in generating conscious perception during visual tasks.

Considering that suppression of neural activity in V1, LM, and AL result in decreased performance on a contrast change detection task[80], and that chapter 3's experiments showed that increased movement and arousal leads to decreased neural response thresholds to contrast in cortical visual areas, insights gleaned from mice suggest that the impact of arousal on neural response gain might constitute a mechanism for improving perception and visual performance when stimulus information is weak.

Notably, chapter 2 discusses a study in which fMRI recordings in head-fixed mice have provided evidence that during an awake state (as compared to under anesthesia), mouse brain networks are functionally organized such as to maximize inter-area information sharing[93], a finding that is consistent with the Global Neuronal Workspace Theory of Consciousness, which posits that a stimulus is perceived when if and when its information is broadcast to many brain areas at once.

Also discussed in chapter 2, Claar et al.[94] performed an EEG and neuropixel study in mice that used the perturbational complexity index (PCI), which was developed by proponents of the Integrated Information Theory of Consciousness in order to assess human consciousness under clinical conditions. Claar et al. found that not only does the PCI capture degree of consciousness in mice, but that a component of EEG signals associated with conscious awareness in mice are dependent on thalamic bursting, suggesting a role for a sub-cortical region in transitioning from unconsciousness to conscious awareness. This study lends further support that PCI is a good measure of consciousness in both mice and primates.

Finally, a study by Kitazono et al.[95] discussed in chapter 2 involved analysis of a whole-brain mouse connectome to determine whether subnetworks underlying conscious states are

recurrent. Researchers found that during awake states (but not during deep sleep or under anesthesia) there are network ‘cores’ with strong bi-directional connections consisting of regions of the cortex, claustrum, and thalamus, with no cores identified in regions thought to be unrelated to consciousness (i.e. cerebellum). These results are consistent with the Recurrent Processing Theory of Consciousness. The experimental results summarized in chapter 4 outline a path forward in using genetic tools in mice to reveal the role of bi-directional circuits (such as those involved in thalamocortical loops) in generating perception.

In conclusion, the research contained in this dissertation demonstrates that mice are an ideal model organism for the study of consciousness, that behavior state impacts neural thresholds, and that activity in early visual regions is likely sufficient for conscious perception in mice. While the current leading theories of consciousness are not mutually exclusive, and mice are not “little primates” - mechanistic insights gleaned from their usage can help build more complete and accurate theories of consciousness that will ultimately solve one of neuroscience’s most fundamental puzzles: how the brain generates conscious awareness.

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