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Yuna Lee March 31, 2024

Assessing the Requirement of Mouse Primary Somatosensory Cortex (S1) for Perception of Movement Direction

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An abstract of
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Abstract

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The somatosensory system, a network of neurons crucial to our sense of touch, enables us to recognize objects, discern textures, and integrate sensory-motor feedback. Specifically, the generation of directional perception in touch is essential to our understanding of the world around us by providing aspects of our spatial awareness, pain localization, survival instincts, and motor control; yet our understanding of how computations of sensory inputs to generate directional selectivity are carried out in the somatosensory system remains underexplored. Preliminary research has suggested that a significant role of the somatosensory cortex is to extract higher order features of tactile stimuli such as directional perception from peripheral somatosensory afferent signals. Here, we use optogenetic silencing on VGAT-ChR2 mice trained to behaviorally discriminate tactile direction on the forepaw to assess the necessity to which forepaw somatosensory cortex (FS1) is needed for directional perception. Specifically, we found that photoinhibition of FS1 resulted in a noticeable decrease in discrimination performance in VGAT-ChR2 mice compared to the photoinhibition of other areas. While this decrease did not reach the threshold of statistical significance, the observed trend indicates a potential influence of FS1 activity in directional perception. This project aims to discern the areas involved with computing higher order features of tactile stimuli to gain a deeper understanding of sensory processing. By doing so, further progress can be made to help individuals with neurological disorders that affect tactile sensation processing.

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Introduction

Directional Selectivity

The generation of directional perception by certain sensory systems is essential to our understanding of the world around us. Its importance may be most obvious and thus most thoroughly explored in the visual system, where directional selectivity (a property used to describe a neuron that responds more to one direction over another) contributes to our perception of motion used to navigate or track moving objects (Freeman 2020). While the exact underlying mechanism for visual directional selectivity is contentious (Y. J. Kim et al. 2022, Freeman 2020), one model that describes the computations underlying directional selectivity in primates identify processing areas in the visual cortex as highly specialized for visual motion (Born and Bradley 2005). In accordance, thalamocortical models suggest that directional selectivity develops de novo in the visual cortex from the integration of inputs from the geniculate thalamic inputs (Rasmussen and Yonehara 2020, Lien and Scanziani 2018). In mice, directionally selective neurons have been shown to be abundantly distributed throughout the primary visual cortex alongside retinal ganglion cells that exhibit directional selectivity (Rochefort et al., 2011). A conclusion put forth is that in mice visual directional selectivity is refined at the cortical level allowing for more narrow directional tuning (Elstrott et al., 2008). Directional selectivity in the somatosensory system may arise similarly, where neurons in the primary somatosensory cortex (S1) respond to more complex stimulus properties than neurons in early subcortical regions.

Evidence for Directional Selectivity in Primary Somatosensory Cortex (S1)

Our sense of touch is generated by the somatosensory system- a network of neurons crucial for tactile perception such as object recognition, texture discrimination, and sensory-motor feedback (Abraira & Ginty, 2013). At the first level of this system are sensory neurons such as low-threshold mechanoreceptors (LTMRs) which initiate the process of tactile perception by converting light, innocuous force stimuli into neural signals (Handler & Ginty, 2021). In mice, the axons of LTMRs relay signals from the forepaw to the central nervous system both at the level of the dorsal horn of the spinal cord and the cuneate nuclei. From these structures, signals are sent to the contralateral ventral-posterior lateral (VPL) nuclei in the thalamus en route to S1, which processes and integrates tactile information for the high-level perception of tactile stimuli (Borich et al., 2015). It has recently been demonstrated that signals

from distinct types of LTMRs are extensively integrated and transformed prior to reaching the cortex (Emanuel et al., 2021; Chirila et al., 2022). We hypothesize that this integration occurs in order to facilitate extraction of higher order features that are not encoded in single LTMRs from the tactile environment. Examples of higher order features include orientations of edges, roughness, and the direction or speed of moving stimuli. A number of studies in primates have demonstrated the role of S1 in the categorization of such higher order features. For example, lesions of the primary somatosensory cortex in monkeys were shown to significantly degrade performance in speed categorization of moving tactile stimuli on glabrous skin with no change in reaction and movement time, indicating a rather specific role of S1 for the perception of higher order features (Zainos et al. 1997). Individual neurons in S1 of monkeys have been reported to exhibit directional selectivity (Costanzo and Gardner 1980; Whitsel, Roppolo, and Werner 1972). A detailed study by Pei et al. (2010) found a population of neurons in the macaque somatosensory cortex to be highly sensitive to the direction of stimulus motion across the fingertips. This sensitivity to direction was conserved regardless of the spatial properties of the stimulus (scanned bars, dot patterns, and random dot displays), indicating that S1 encodes a strong representation of motion direction (Pei et al., 2010).

Optogenetic S1 Silencing to Determine its Role in Directional Perception

While many studies have investigated S1 and its role in sensory perception in primates, most research on the somatosensory system in mice has been conducted on the whisker system (O'Connor, Krubitzer, and Bensmaïa 2021). Each whisker on the snout is individually represented in the barrel cortex by a brain structure in the somatosensory cortex called a barrel (Petersen, 2019). When a whisker is stimulated, sensory information signals through the brainstem and thalamus to the cortex, where cortical neurons encode more complex stimulus properties (Bale & Maravall, 2018). Particularly, a variety of excitatory neurons in the barrel cortex are found to be selective for stimulus features such as whisker deflection angles (Lavzin et al., 2012) and object location (Cheung et al., 2020; Sofroniew et al., 2015), which are not encoded directly by the sensory neurons. Directional selectivity in the mouse barrel cortex was found to reflect angular selectivity throughout the whisker-to-barrel pathway, with GABA-ergic interneurons further shaping selectivity through inhibition (Guy et al., 2023). While the mechanisms of directional selectivity for vision and whisker stimuli have been explored, to our

knowledge, no studies have investigated directional selectivity within S1 of mice for the glabrous skin of the forepaw.

In this study, we investigated the extent to which S1 is needed for directional discrimination of a light-touch stimulus applied to the glabrous skin of the forepaw using VGAT-ChR2 mice. Vesicular GABA Transporter (VGAT) is a protein that is responsible for the accumulation of GABA in GABAergic (inhibitory) neurons while channelrhodopsin-2 (ChR2) is a light-sensitive rhodopsin that originated from unicellular green algae (Boyden et al., 2005). Through the use of bacterial artificial chromosome (BAC) transgenic strategy, VGAT-CHR2 mice are genetically modified so that all GABAergic neurons in the brain express the excitatory opsin ChR2, allowing for these neurons to be activated in response to short-wavelength light (Zhao et al., 2011). This activation of the inhibitory GABAergic neurons causes indirect neuronal inhibition of surrounding neurons, effectively silencing the region of interest (Babl et al., 2019). This optogenetic technique enables a genetically based high-temporal resolution method to control neural activity noninvasively and allows for the targeted silencing of multiple brain regions (Guo et al., 2014). Chronic methods of silencing such as lesions may confound the role S1 has on the behavioral deficits being measured as rewiring or compensation could occur during recovery periods (Hong et al., 2018), and pharmacological methods would not allow for silencing of multiple cortical regions within a single behavioral session. Transient silencing through the use of optogenetics allows for independent perturbations of different cortical areas in S1 within a single session for each mouse.

Therefore, we used transient optogenetic silencing of cortical activity to determine if forepaw somatosensory cortex (FS1) and nearby cortical regions are needed for directional discrimination. The use of transgenic mice enables us to go beyond neural recordings by allowing experimental control of neural activity in targeted cortical regions, providing a mechanistic understanding of the brain regions involved in tactile perception. The perception of directionality on our skin by the somatosensory system is a component of many aspects of the sensations we experience daily, including spatial awareness, pain localization, our survival instincts, and motor control. While the goal of this experiment is to enhance our basic understanding of neurobiology, the implications that build upon the knowledge we will gain may lead to solutions and applications for neurological disorders with sensory processing difficulties,

such as Parkinson's disease. As such, exploring the fundamental structure and function of tactile feature representation in S1 can yield valuable information for our understanding of the somatosensory system and build a basis on which further research can be done.

Methods

Mice Model

Experimental procedures were approved by the Emory Institutional Animal Care and Use Committee (IACUC; PROTO202200102) and were carried out in accordance with the outlined standards. Six 4-month-old VGAT–ChR2 mice were used in this study. In this paper, individual mice are referred to with their identifiers as BBR1, BBS2, BBS4, BBQ2, BBQ3, and BCM2. BBR1, BBS2, and BBS4 are male mice while BBQ2, BBQ3, and BCM2 are female mice. Female mice were trained by another experimenter, Ruorong Qi. Mice had free access to food but were water restricted one week prior to and throughout the behavioral training to incentivize behavioral performance. Mice were given an allowance of 40 mL/kg of water daily, which was gained through performance of the behavioral task and supplementation after behavioral sessions as needed. Weight and health conditions of the mice were monitored daily.

Surgical Procedures: Optical Window Implantation

To determine the requirement of S1 for directional discrimination in mice, a glass cranial window and headplate was surgically implanted in the skull above right S1 and the mice were given one week of rest before habituation and training was started. The transparent optical window allows S1 and neighboring cortical regions to be exposed to light for photoinhibition. The headplate is secured to the skull of the mouse with dental cement, allowing for the head to be immobilized while behavioral and photoinhibition sessions are conducted. The 3-mm diameter window was centered 1.65 mm lateral and 0.4 mm posterior to bregma to encompass hindpaw and forepaw regions of S1 as well as the posterior parietal cortex.

Optogenetics

A 445-nm laser beam controlled by a National Instruments (NI) USB-6341 device coupled to a 2D galvo mirror system was used to focus light onto precise coordinates upon the cortical surface. Laser frequency and pulse was set to 40 Hz and 0.5 ms, respectively (average power of 5 mW) for appropriate cortical silencing based on past studies using similar optogenetic silencing strategies (Li et al. 2019, Guo et al., 2014). Based on a similar study that stimulated another region of S1 (the barrel cortex) in VGAT-ChR2 mice, the spatial resolution of the photoinhibition is estimated to have a 0.9 - 1.0 mm radius (~1.8-2.0-mm diameter) surrounding the point of illumination (Guo et al., 2014). This effective-silencing radius is sufficient to encompass all of the forepaw S1, which is ~1.5 mm across its largest axis (Cases et al., 1996).

During photoinhibition trials, photoinhibition of the cortex was applied on 30% of the 400 trials in each session: 10% to the experimental forepaw S1 (FS1), 10% to hindpaw S1 (HS1), and 10% to the posterior parietal cortex (PPC), with a randomized trial order. The remaining 70% of trials had the laser light directed to the dental cement that secures the headplate to the skull. The coordinates for each cortical area were derived from past studies and were used to direct the laser to its appropriate location after being centered on the window: FS1 located 0 mm posterior and 2.1 mm lateral to bregma, HS1 located 0.6 mm posterior and 1.65 mm lateral to bregma, and PPC located 1.7 mm posterior and 1.5 lateral to bregma (Figure 1A). Photostimulation was delivered from 0.1 seconds before the start of the stimulus to 0.1 seconds after the end of the stimulus to encompass the full stimulus duration (Figure 2A).

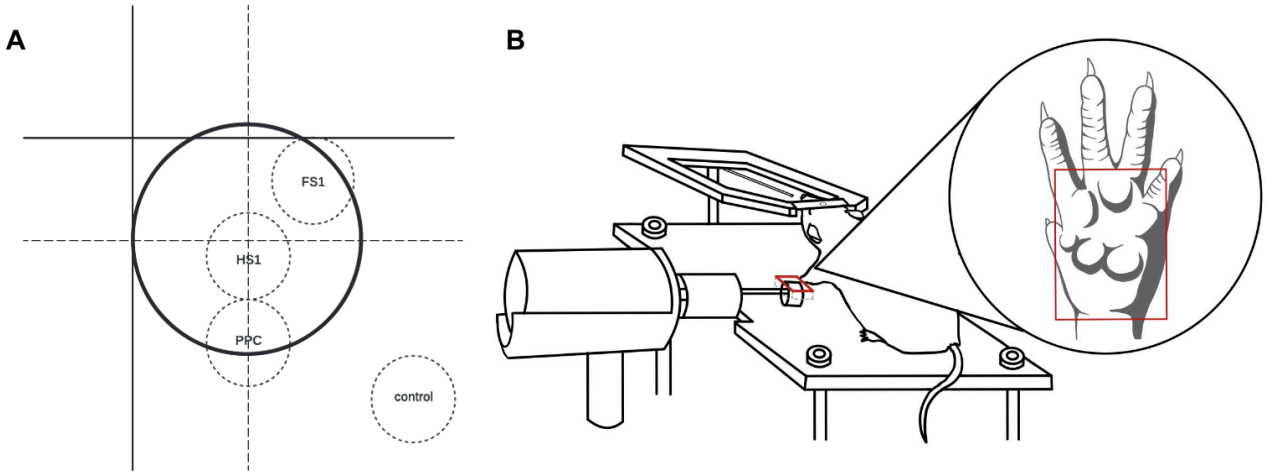


Figure 1. Behavioral Setup. A) Schematic of photoinhibition spread on FS1, HS1, PPC, and control in reference to the cranial window as represented by the circle, and bregma as represented as solid cross (Emanuel et al., 2021, Arlt et al., 2022). B) Schematic of behavioral training setup. Red outline indicates area where glabrous skin of forepaw contacts with stimulus brush (image of forepaw adapted from BioRender).

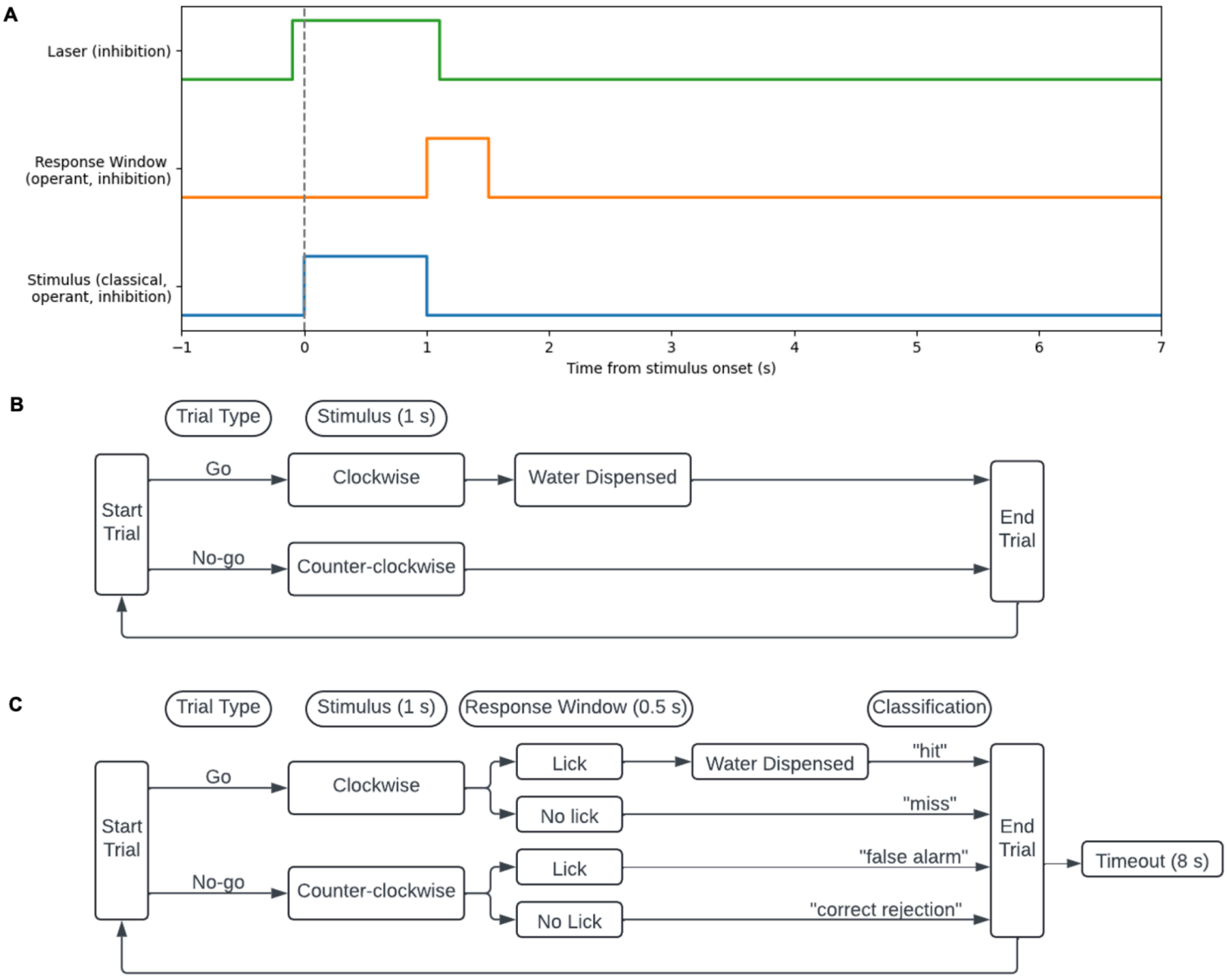


Figure 2. Trial Structures. A) Onset and duration of stimulus, response window, and laser. Dotted gray line indicates the end of the trial. B) Block diagram of possible events in a single trial for classical conditioning (trial duration = 7 s). C) Block diagram of possible events in a single trial for operant training (trial duration = 7 s).

Stimulus and Apparatus Construction

To test the directional selectivity of S1, a stimulus tailored to this examination should be consistent in all variables excluding the direction of movement. Therefore, a motor-controlled, wool-covered circular brush was selected as the stimulus. A brush is particularly suitable as it

allows for the cutaneous surface of the skin and stimulus to be in motion with respect to one another through mechanical friction (Whitsel, Roppolo, and Werner 1972). Through the use of soft wool fabric to cover the brush, the stimulus was designed to recruit LTMRs, which by definition are activated by weak, innocuous mechanical force (Abraira & Ginty, 2013). The brush rotates clockwise or counter-clockwise (distal to proximal or proximal to distal in reference to the mouse) to present two distinct directions for the mice to discriminate. Using a brush programmed to generate the same pressure, time interval, and speed separate from the directionality contributes to a controlled experiment. The nature of the brush also allows us to circumvent the problem of an inherent pixelation that might be felt if probes were used. To implement the stimulus for the behavioral trials, the motor was connected to an Arduino Uno, a microcontroller that controls the speed and direction of the motor with precise timing. The Arduino Uno was connected to a NI USB-6341 DAQ, which was used to define the trial structure and collect timestamps of when licks are detected by the lick sensor. Code for stimulus and behavioral control was written in Python and C.

When performing behavioral trials, the mouse's head was immobilized by their headplate to ensure stability of the head while undergoing photoinhibition that requires precise alignment with the targeted brain region. The lickspout was positioned in front of the mouth. The brush stimulus was applied to the ventral surface of the left forepaw through a 3.7-mm by 4-mm opening in the platform (Figure 1B). The paw was tethered over the opening to immobilize it and to ensure consistent application of the stimulus throughout the session. White noise was played during all behavioral training sessions to mask the auditory profile of the brush stimulus motor. For photoinhibition trials, the laser beam device was mounted ~160 mm above the apparatus to focus the laser pulses on the surface of the cortex. Before each photoinhibition session, the laser position was calibrated to the center of the optical window so that FS1, HS1, and PPC could be targeted for silencing.

Behavioral Paradigm

A head-fixed behavioral paradigm with go/no-go tasks was used to facilitate precise behavioral monitoring and neural recordings. All habituation and training was done in the dark. Mice were first habituated to head- and paw-fixation (left paw) for 3 days, 1 session per day for 30 minutes. Then, classical conditioning sessions were conducted for ~3 weeks, with 5 sessions each week for 1 hour. During these sessions, water was dispensed through the lick spout after

one stimulus direction but not the other (Figure 2B). Each water reward was calibrated to dispense 0.0041 mL, an amount chosen to ensure that the water remained on the tip of the lick spout, allowing the mouse to detect and lick in response. The purpose of classical conditioning is to facilitate the association between the positive reinforcement, water, and one direction (Go trial).

Operant conditioning was then conducted to assess whether the mice were able to report the correct stimulus direction by licking the water spout (Figure 2C). The trial structure remained the same for both directions, but with a response window following the stimulus with four possible outcomes: hit, miss, correct rejection, and false alarm. A hit occurred when the mouse licked in response to the targeted direction; a miss occurred when the mouse did not lick in response to the targeted direction. A correct rejection occurred when the alternate direction was given and the mouse did not lick; a false alarm occurred when the alternate direction was given and the mouse licked. All the licks that register for the four possible outcomes must be within the 0.5 second response window that followed the stimulus. Contrary to classical conditioning, during operant conditioning, the water reward was only dispensed if the mouse licked within the response window for the correct direction. An 8-second timeout punishment was also introduced with each false alarm (i.e., when a lick was detected during the response window during a No-go trial). The operant conditioning paradigm allows for the licks to be indicative of discriminatory learning between the two directional stimuli.

Performance was evaluated through the calculation of d prime (d'), a measure of sensitivity used to indicate the detector's ability to discriminate between signal and noise (Botella & Suero, 2019). The formula for d' is as follows: $d' = z(H) - z(FA)$, where H and FA are the hit rate and false alarm rate, respectively. These rates are Z-transformed to standardize the comparisons on a common scale. Large positive d' values are indicative of better discrimination ability compared to a low d' value. We counterbalanced the target and alternate stimuli by assigning three mice (BBS2, BBS4, BBQ3) to be rewarded for the clockwise direction and the other three mice (BBR1, BBQ2, BCM2) to be rewarded for the counterclockwise direction throughout all behavioral sessions. To sculpt behavior, we implemented forced no-go trials when the previous 20 trials had a false alarm rate greater than 0.9. In each session, if the mouse remained unresponsive (no licks) to the rewarded stimulus for greater than ~5 continuous trials, the session was ended. This measure was based on the assumption that the mouse was no

longer motivated to respond due to being satisfied with the water rewards received earlier in the session.

Results

Mice were able to reliably discriminate direction above chance levels

Mice (n=6) were trained in the dark through a series of classical and operant conditioning sessions on a head-fixed Go/No-go tactile discrimination task. During classical conditioning, mice were able to detect and consume water from the lick spout, as indicated by the multiple licks after water was dispensed (Figure 3A). By operant conditioning, the mice associated the Go stimulus with the water reward as indicated by licks that registered during the stimulus before the response window opened for the opportunity for water to be dispensed (Figure 3B). Go trials were rewarded with water if the mouse licked within the response window (0.5 s, starting at the end of the stimulus) while a lick in this window in No-go trials resulted in a time-out (8 s) punishment following the end of the trial. Following 10 sessions (~400 trials per session, one session per training day) of behavioral training, d' gradually increased to values well above chance for the male mice BBR1, BBS2, and BBS4 (Figure 3C). A value of d' = 0 is considered chance performance. Once mice reached an expert level (d' > 2, threshold for expert level), we began photoinhibition trials. Although the d' of female mice was reliably above chance levels (d' = 0), they did not reach an expert level of learning (d' > 2) so photoinhibition trials were not performed on them. Additional no-contact sessions for the male mice were conducted later on in the experiment to confirm the intended stimulus was what drove behavior, rather than possible extraneous variables the mice may rely on such as the motor sound or vibrations made by the motor. This was done by lowering the motor so that no contact was made between the brush and the paw. No-contact sessions were then run by conducting operant conditioning sessions with the lowered motor, and the performance for each mouse without contact with the brush was near chance (Figure 3D). Overall, through classical and operant conditioning, mice were able to reliably learn to report the direction of the brush.

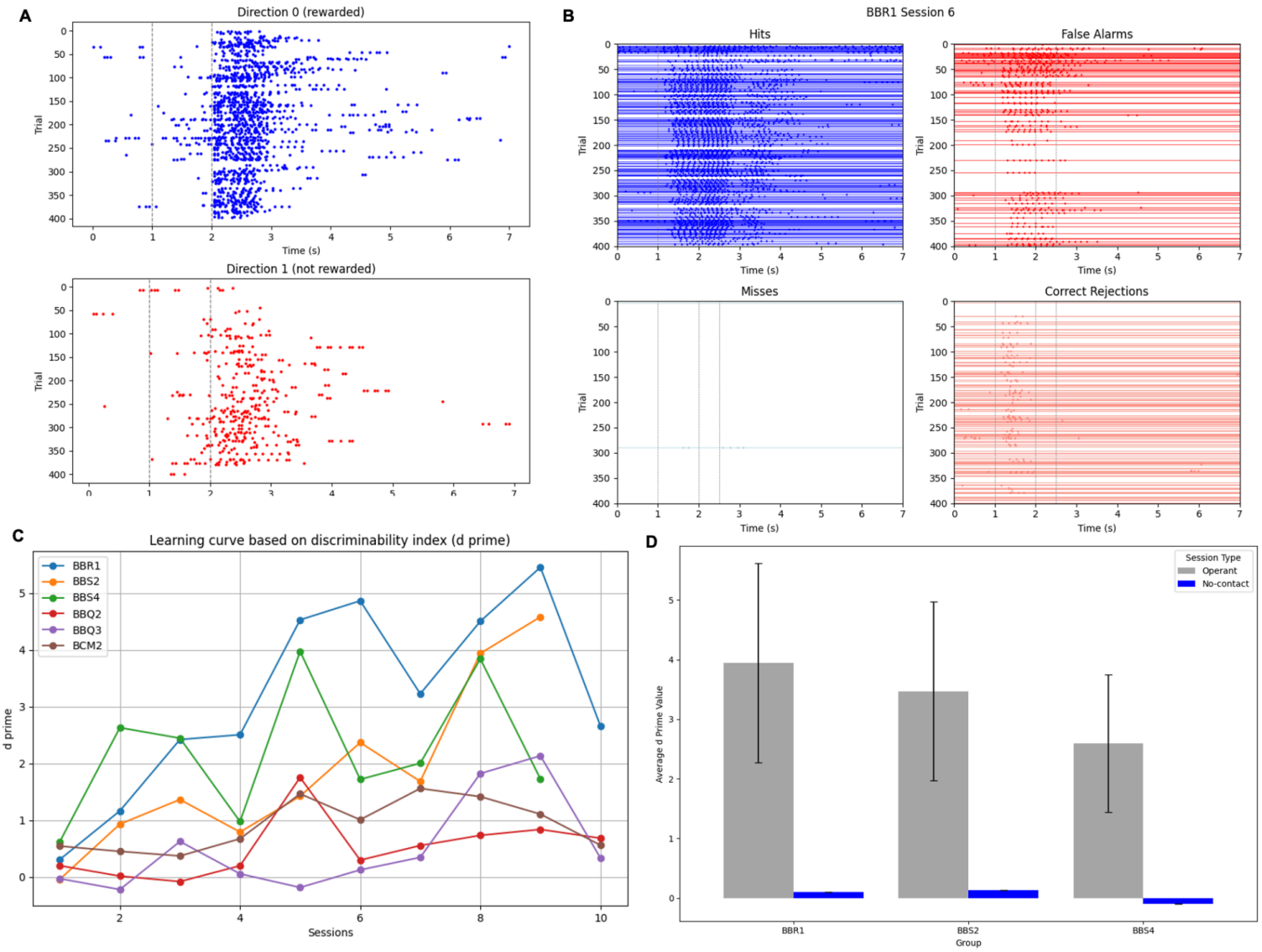
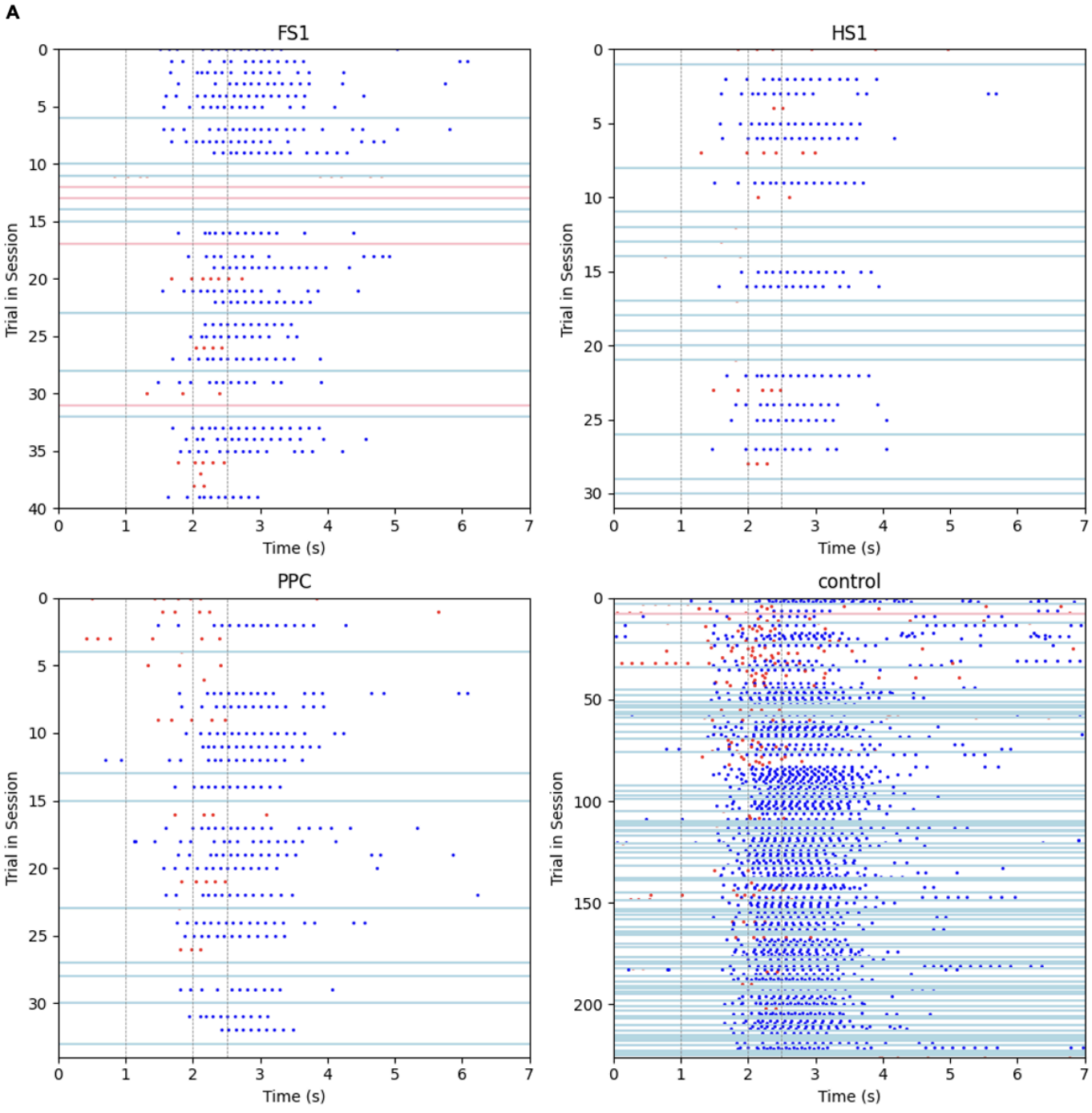


Figure 3. Mice were able to reliably report direction. A) Example of lick raster for one classical training session. Top graph represents all licks registered when the go-stimulus was run. Bottom graph represents all licks registered when the no-go stimulus was run. Vertical dashed lines indicate onset and offset of stimulus. Each dot represents a lick registered from the lick sensor (Session 11, BBR1). B) Example of Lick raster for one operant training session. Each colored bar represents the trial classification according to the four possible outcomes: hit, miss, false alarm, and correct rejection. Each dot represents a lick registered from the lick sensor (Session 6, BBR1, d' = 4.86265). Dark blue indicates "hit", light blue indicates "miss", red indicates "FA", and salmon indicates "CR" . C) Learning curve based on discriminability index

(d') over operant conditioning sessions prior to photoinhibition trials. D) Averaged d' of operant sessions compared to no-contact sessions.



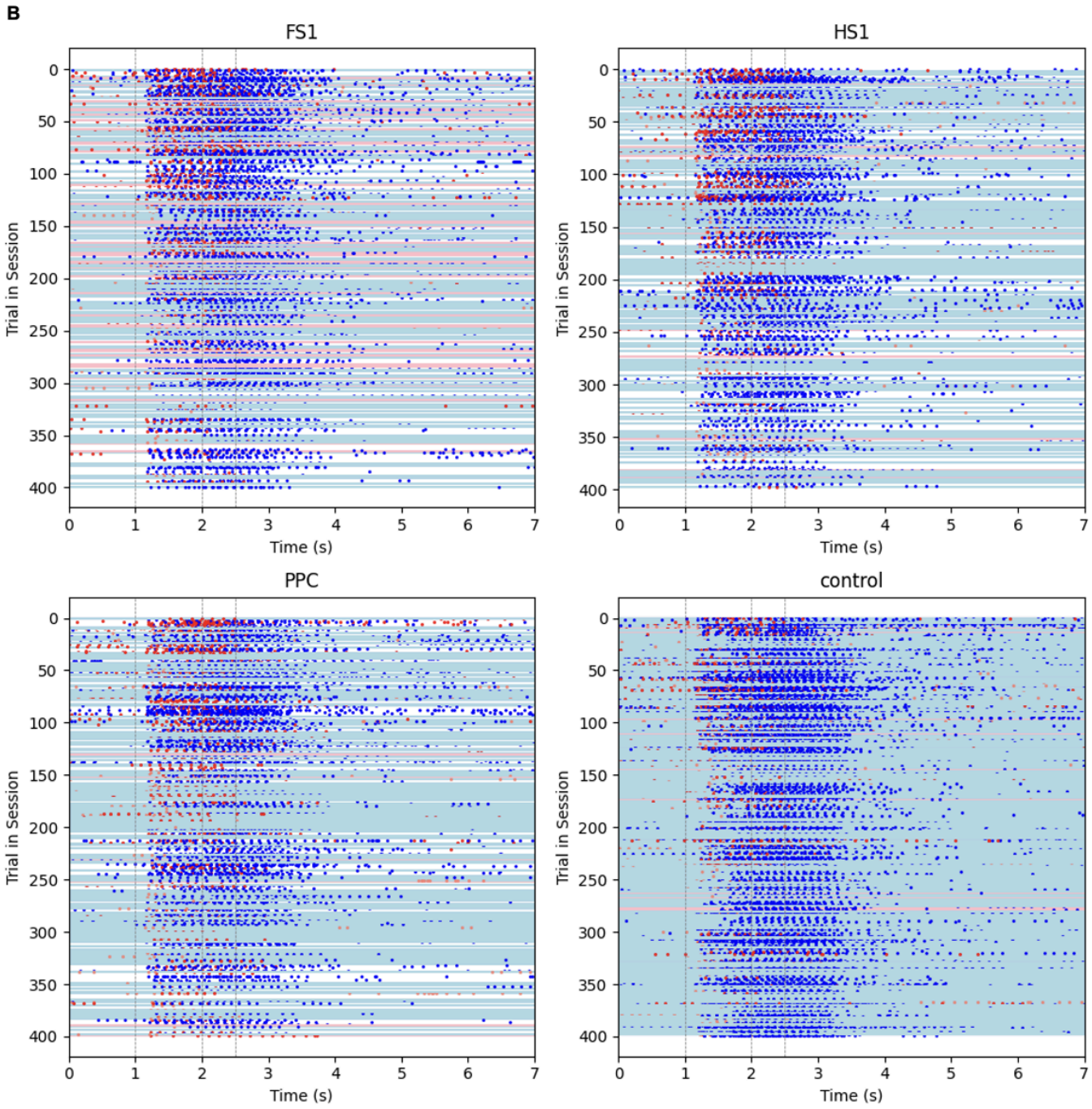


Figure 4. Lick rasters by photoinhibition region. A) Lick raster of one photoinhibition session (session 7, BBS4) Each dot represents a lick. Color indicates trial classification according to the four possible outcomes: hit, miss, false alarm, and correct rejection. Dark blue dots indicate "hit", pink lines indicate "miss", red dots indicate "FA", and light blue lines indicate "CR" . B) Lick raster for aggregated photoinhibition sessions for all mice with the same color classification as A).

Analysis of lick rasters by photoinhibition region

Once mice (n=3) were able to reliably and consistently discriminate the directions, sessions with photoinhibition were conducted to assess the effects of silencing of FS1, HS1, and PPC during the stimulation presentation on mouse performance. Photoinhibition trials were collected from a minimum of seven sessions, with approximately 400 trials per session (Figure 4A). To determine if d' is solely influenced by the effects of optogenetic silencing rather than changes in motivation, aggregated lick rasters of all photoinhibition sessions divided by silenced regions are visualized (Figure 4A). Response latency as measured by a delay in licks after stimulus onset may indicate that d' was impacted by changes in motivation (Spangenberg & Wichman, 2018). However, no difference of lick onset can be visually discerned when comparing the lick rasters of all regions. Instead, there were a notable number of missed trials in the FS1 graph compared to HS1 and PPC. Indeed, when the total number of missed trials was calculated for each area, FS1 had a higher occurrence of missed trials compared to other areas (Figure 4B; percentage of missed trials: FS1- 7.10%, HS1- 1.68%, PPC- 1.14%, control- 1.59%).

Transient silencing of FS1 has no significant difference on d' compared to control and other areas

To evaluate the role of cortical regions in the discrimination of directions, transient silencing of FS1, PPC, HS1, and control was performed on separate trials within each photoinhibition session. The d' for each area was calculated from 7 photoinhibition sessions. No significant difference was found (H-statistic = 2.0, p value = 0.36788), suggesting that there was no discernible impact of cortical inhibition across the silenced regions with this sample size. However, it is worthy to note that the sample size was small, so this statistical test is likely underpowered. While not statistically significant, the d' during FS1-silenced trials across sessions for each mouse tended to be smaller than the other silenced regions and controls (Figure 5A). When the d' for all areas are averaged across all mice and sessions, FS1 is also seen to have the lowest d', followed by PPC (Figure 5B).

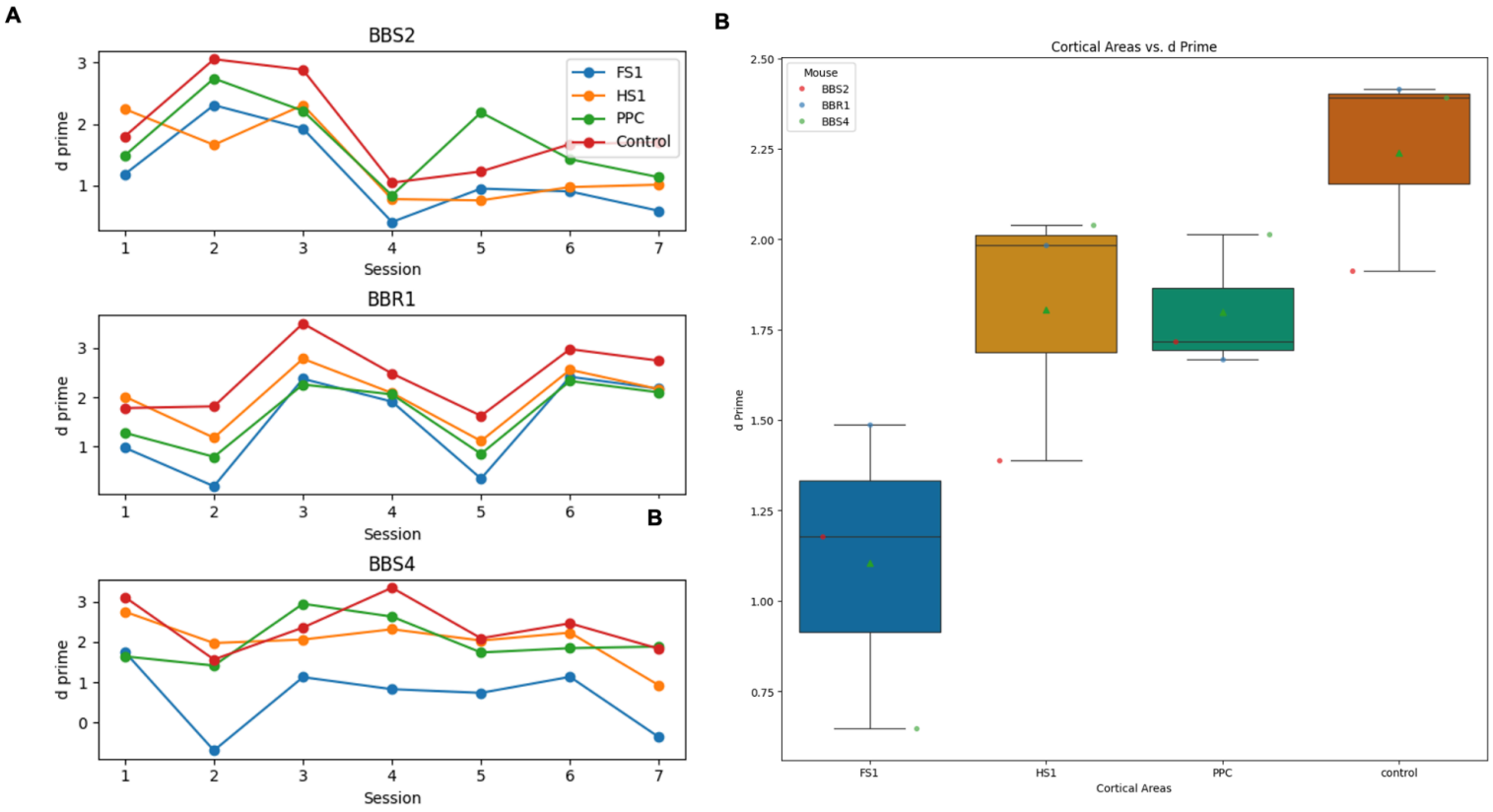


Figure 5. Silencing effects on behavioral discrimination. A) Learning curve based on discriminability index (d') of FS1, HS1, PPC, and control over photoinhibition sessions for each mouse. B) Boxplot illustrating the distributions of d' across each mouse for FS1, HS1, PPC, and control areas. The d' for each area from each session is displayed as a dot, with the mean indicated by the green triangle and the median indicated by a horizontal line. Whiskers represent the minimum and maximum of the dataset and extend within 1.5 times the IQR from the box edges. The bottom half of the box represents the first quartile and the top half represents the third quartile.

Statistical Analysis

To analyze differences between the areas of optogenetic inhibition, the Kruskal-Wallis ANOVA test was applied across three mice for each silenced region (FS1, HS1, PPC) and control, revealing no significant difference across the areas ($H[2, 3] = 2$, $p = 0.37$). Given the relatively small sample size (n=3) that may have impacted the power of the analysis, a simulation-based power analysis for a paired comparison between FS1 and control was then

performed to determine the sample size needed to see a significant difference. A threshold of 0.8 was set for the estimated power to surpass, with each simulation iteration increasing the sample size for each Wilcoxon signed-rank test until the threshold was met. This analysis revealed a sample size of 10 needed to reach an estimated power of 0.811.

Discussion

In this experiment we assessed the role of S1 in directional discrimination, a fundamental aspect of tactile perception. Employing optogenetic techniques, S1 was transiently silenced in trained VGAT-ChR2 mice (n=3) to assess its necessity in directional perception. While transient silencing of FS1 compared to other S1 areas and control did not result in a significant difference, a trend toward lower d' for this area was observed, warranting further study and experimentation. Particularly, FS1 having the lowest d' average across all areas may indicate that by increasing the sample number of mice being tested, a statistical difference between the control area may be observed.

Further experimentation can be done to determine if FS1 is necessary for mice to perceive and discriminate direction. It is important to note certain limitations in the experimental design that may influence interpretations of results. The low sample size (n=3 mice) limits the statistical power of analysis, and the use of only male mice in the photoinhibition trials could limit the generalizability of findings. 3 female mice were also included in the study, but did not undergo the photoinhibition sessions due to a slower acquisition in discrimination learning during the classical and operant trials. This deviation from the male mice may be attributable to the difference in experimenters handling and running the training. It may be possible that the techniques in which each experimenter conducted the behavioral sessions may have led to a difference in outcome. Additionally, the behavioral sessions for the female mice were more frequently cut short compared to the male mice due to either decreased motivation or earlier satiety of water. Lowering the amount of water dispensed for the female mice could increase the amount of trials run, which could result in a better performance over time. A question on if light touch was used as the tactile perception to discriminate directions could also be posed. While care was taken to ensure that the stimulus does not exert the significant pressure or movement that engages proprioceptors, there is a possibility that mice could also be using proprioception via the walking pads on their forepaw to indicate a difference in stimuli rather than light touch.

While this study showed that the silencing of FS1 had the lowest d', it is worthy to note that the mean d' for both PPC and HS1 trials were lower than control for all but the first session during photoinhibition trials (Figure 3A). HS1 responds to tactile indentations on the hindpaw glabrous skin while PPC is implicated to be involved in decision-making and mediation between sensory to motor commands (Emanuel et al., 2021; Pho et al., 2018). In this study

decision-making took place when mice were conditioned to lick for one direction over the opposite direction. The lack of significant difference in d' between PPC and control may indicate that FS1 could suffice for the mice to both detect and behave in accordance with their training. While the absence of a significant difference does not necessarily imply the absence of contribution from the PPC, it is possible that the role of PPC can become more evident under more complex conditions where different sensory inputs are being processed simultaneously. PPC is also positioned between sensory and prefrontal areas, but may not be necessary in the process of sensory information as mouse OFC receives direct projections from S1, and is involved with learning simple Pavlovian acquisitions (Lyamzin & Benucci, 2019; Panayi & Killcross, 2021). PPC has also been shown to play a central role in learning multimodal contingencies and detecting novel stimuli, a concept that was not explicitly tested for in this study (Lyamzin & Benucci, 2019). Additionally, this experiment used passive touch, where the tactile stimulus is passively applied on the skin, as opposed to active touch, where voluntary movement is used to contact the stimulus (Watanabe et al., 2020). While motor output demonstrated by licking was observed in this study, learning with active touch may increase the significance to which PPC is needed with subsequent discrimination of tactile direction as the method of tactile input can activate different areas of the cortex. The effect of photoinhibition as the method for silencing cortical areas could also be considered as a possible explanation for lower d' in all silenced areas; for example, the use of VGAT-ChR2 mice allows for transient silencing of the cortex through the activation of inhibitory cells with ChR2, but due to the axonal arborizations of GABAergic interneurons, long-range connections could be stimulated, both subcortically and laterally (Babl et al., 2019). Given that FS1 may play a role in directional discrimination, potential long-range lateral photoinhibition to FS1 from HS1 or PPC could explain the decrease in d' when those regions were stimulated.

Future directions

Additional experiments in the future should be run to verify experimental findings, such as running the experiment on wild-type mice to verify that the impairment of discrimination performance was due to the silencing of cortical regions, and not a nonspecific effect of laser stimulation of brain tissue. Post-behavioral verifications, such as verifying the accuracy of silenced areas, should also be conducted. This can be done by removing the cranial window to record targeted cortical areas using a multielectrode array while stimulating the corresponding

body area (e.g. recording from FS1 while lightly brushing the forepaw). Using a trial structure that implements a“delay epoch” between the stimulus and response window could help delineate perceptual decision-making by separating the tactile perception (sensation) with the licking behavior (action) (Guo, Hires, et al., 2014).

Many avenues of future studies can stem from this project to further understand light touch in the somatosensory system. For example, while silencing of FS1 has been observed alongside a decreased d’ trend through this experiment, its requirement for learning directional discrimination remains untested. The use of various silencing techniques may reveal discrepancies between the role FS1 plays in sensation and learning; such nuance was demonstrated when the ablation of S1 was shown to have no effect on task acquisition while transient optogenetic silencing of the same area impaired detection behavior (Hong et al., 2018). More research can also be done with an emphasis on decision-making that requires the perception of directional discrimination. This can be done by increasing the complexity of the tasks (having the mice associate sequences of directions to a reward) or introducing a memory component to the task (having a delay in between stimulus and response window) the mice undertake to perform well. One study on the barrel cortex and other cortical areas used photoinhibition to uncover which areas were involved in tactile decisions involving active whisker movement (Guo, Li, et al., 2014). Similarly, the task flow from directional discrimination to decision making can be examined. A finer analysis into the cell types that play a role in cortical integration of sensory stimuli for directional selectivity in FS1 could also produce interesting findings. Directional selectivity in GABAergic interneurons were found through the examination of the whisker to barrel pathway, suggesting GABAergic inhibition as a modulator for feature selectivity (Guy et al., 2023). Exploring the different neuronal types that may exhibit directional selectivity in FS1 would deepen our comprehension of the cellular mechanisms that are involved with higher-order perceptions of tactile stimuli.

Overall, continued research into the computational systems governing light touch sensory perception in mice offers a pathway to a deeper understanding of sensory systems. By further investigating how mice perceive direction and other intricate aspects of somatosensory stimuli, insight into the basic mechanisms of tactile signal processing can be gained. This knowledge could ultimately guide the development of improved treatments and applications for sensorimotor dysfunctions (Borich et al., 2015). Therefore, exploration into this field not only

enriches our comprehension of the somatosensory system but also holds potential to address and alleviate related dysfunctions.

References

Abraira, V. E., & Ginty, D. D. (2013). The sensory neurons of touch. Neuron, 79(4), 618–639. https://doi.org/10.1016/j.neuron.2013.07.051

Arlt, C., Barroso-Luque, R., Kira, S., Bruno, C. A., Xia, N., Chettih, S. N., Soares, S., Pettit, N. L., & Harvey, C. D. (2022). Cognitive experience alters cortical involvement in goal-directed navigation. eLife, 11. https://doi.org/10.7554/elife.76051

Babl, S. S., Rummell, B. P., & Sigurdsson, T. (2019). The spatial extent of optogenetic silencing in transgenic mice expressing channelrhodopsin in inhibitory interneurons. Cell Reports, 29(5), 1381-1395.e4. https://doi.org/10.1016/j.celrep.2019.09.049

Bale MR, Maravall M. Organization of sensory feature selectivity in the whisker system. Neuroscience. 2018 Jan 1;368:70-80. doi: 10.1016/j.neuroscience.2017.09.014. Epub 2017 Sep 14. PMID: 28918260; PMCID: PMC5798594.

Borich MR, Brodie SM, Gray WA, Ionta S, Boyd LA. Understanding the role of the primary somatosensory cortex: Opportunities for rehabilitation. Neuropsychologia. 2015 Dec;79(Pt B):246-55. doi: 10.1016/j.neuropsychologia.2015.07.007. Epub 2015 Jul 9. PMID: 26164474; PMCID: PMC4904790.

Born, Richard T., and David Bradley. 2005. "STRUCTURE AND FUNCTION OF VISUAL AREA MT." *Annual Review of Neuroscience* 28 (1): 157–89. https://doi.org/10.1146/annurev.neuro.26.041002.131052.

Botella, J., & Suero, M. (2019). Recovering the variance of d' from hit and false alarm statistics. *Behavior Research Methods*, *52*(1), 1–22. https://doi.org/10.3758/s13428-018-1181-x

Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., & Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. Nature Neuroscience, 8(9), 1263–1268. https://doi.org/10.1038/nn1525

Cases, O., Vitalis, T., Seif, I., De Maeyer, E., Sotelo, C., & Gaspar, P. (1996). Lack of barrels in the somatosensory cortex of monoamine oxidase a–deficient mice: Role of a serotonin excess during the critical period. *Neuron*, *16*(2), 297–307. https://doi.org/10.1016/s0896-6273(00)80048-3

Cheung, J. A., Maire, P., Kim, J., Lee, K., Flynn, G., & Hires, S. A. (2020). Independent representations of self-motion and object location in barrel cortex output. PLOS Biology, 18(11), e3000882. https://doi.org/10.1371/journal.pbio.3000882

Chirila, A. M., Rankin, G., Tseng, S., Emanuel, A. J., Chavez‐Martinez, C. L., Zhang, D., Harvey, C. D., & Ginty, D. D. (2022). Mechanoreceptor signal convergence and transformation in the dorsal horn flexibly shape a diversity of outputs to the brain. Cell, 185(24), 4541-4559.e23. https://doi.org/10.1016/j.cell.2022.10.012

Costanzo, Richard M., and Esther P. Gardner. 1980. "A Quantitative Analysis of Responses of Direction-sensitive Neurons in Somatosensory Cortex of Awake Monkeys." *Journal of Neurophysiology* 43 (5): 1319–41. https://doi.org/10.1152/jn.1980.43.5.1319.

Elstrott J, Anishchenko A, Greschner M, Sher A, Litke AM, Chichilnisky EJ, Feller MB. Direction selectivity in the retina is established independent of visual experience and cholinergic retinal waves. Neuron. 2008 May 22;58(4):499-506. doi: 10.1016/j.neuron.2008.03.013. PMID: 18498732; PMCID: PMC2474739.

Emanuel, Alan J., Brendan P. Lehnert, Stefano Panzeri, Christopher D. Harvey, and David D. Ginty. 2021. "Cortical Responses to Touch Reflect Subcortical Integration of LTMR Signals." *Nature* 600 (7890): 680–85. https://doi.org/10.1038/s41586-021-04094-x.

Freeman, Alan W. 2020. "A Model for the Origin of Motion Direction Selectivity in Visual Cortex." *The Journal of Neuroscience* 41 (1): 89–102. https://doi.org/10.1523/jneurosci.1362-20.2020.

Gilad, A., Gallero‐Salas, Y., Groos, D., & Helmchen, F. (2018). Behavioral strategy determines frontal or posterior location of Short-Term memory in neocortex. Neuron, 99(4), 814-828.e7. https://doi.org/10.1016/j.neuron.2018.07.029

Guo, Z. V., Li, N., Huber, D., Ophir, E., Gutnisky, D., Ting, J. T., Feng, G., & Svoboda, K. (2014). Flow of cortical activity underlying a tactile decision in mice. *Neuron*, *81*(1), 179–194. https://doi.org/10.1016/j.neuron.2013.10.020

Guo ZV, Hires SA, Li N, O'Connor DH, Komiyama T, Ophir E, Huber D, Bonardi C, Morandell K, Gutnisky D, Peron S, Xu NL, Cox J, Svoboda K. Procedures for behavioral experiments in head-fixed mice. PLoS One. 2014 Feb 10;9(2):e88678. doi: 10.1371/journal.pone.0088678. Erratum in: PLoS One. 2014;9(6):e101397. PMID: 24520413; PMCID: PMC3919818.

Guy, J., Möck, M., & Staiger, J. F. (2023). Direction selectivity of inhibitory interneurons in mouse barrel cortex differs between interneuron subtypes. Cell Reports, 42(1), 111936. https://doi.org/10.1016/j.celrep.2022.111936

Handler, A., & Ginty, D. D. (2021). The mechanosensory neurons of touch and their mechanisms of activation. Nature Reviews Neuroscience, 22(9), 521–537. https://doi.org/10.1038/s41583-021-00489-x

Hong, Y. K., Lacefield, C., Rodgers, C. C., & Bruno, R. M. (2018). Sensation, movement and learning in the absence of barrel cortex. Nature, 561(7724), 542–546. https://doi.org/10.1038/s41586-018-0527-y

Kim, Yeon Jin, Barry T. Peterson, Joanna D. Crook, Hannah R. Joo, Wu Jiajia, Christian Puller, Farrel R. Robinson, et al. 2022. "Origins of Direction Selectivity in the Primate Retina." *Nature Communications* 13 (1). https://doi.org/10.1038/s41467-022-30405-5.

Lavzin et al. (2012) Lavzin M, Rapoport S, Polsky A, Garion L, Schiller J. Nonlinear dendritic processing determines angular tuning of barrel cortex neurons in vivo. Nature. 2012;490(7420):397–401. doi: 10.1038/nature11451.

Li, Nuo, Susu Chen, Zengcai V. Guo, Han Chen, Yan Huo, Hidehiko K. Inagaki, Guang Chen, et al. 2019. "Spatiotemporal Constraints on Optogenetic Inactivation in Cortical Circuits." eLife 8 (November). https://doi.org/10.7554/elife.48622.

Lien, Anthony D, and Massimo Scanziani. 2018. "Cortical Direction Selectivity Emerges at Convergence of Thalamic Synapses." *Nature* 558 (7708): 80–86. https://doi.org/10.1038/s41586-018-0148-5.

Lyamzin D, Benucci A. The mouse posterior parietal cortex: Anatomy and functions. Neurosci Res. 2019 Mar;140:14-22. doi: 10.1016/j.neures.2018.10.008. Epub 2018 Nov 20. PMID:

Maria Nolano, Vincenzo Provitera, Anna Estraneo, Mona M. Selim, Giuseppe Caporaso, Annamaria Stancanelli, Anna Maria Saltalamacchia, Bernardo Lanzillo, Lucio Santoro, Sensory deficit in Parkinson's disease: evidence of a cutaneous denervation, Brain, Volume 131, Issue 7, July 2008, Pages 1903–1911,30465783.

Nolano, M., Provitera, V., Estraneo, A., Selim, M. M., Caporaso, G., Stancanelli, A., Saltalamacchia, A. M., Lanzillo, B., & Santoro, L. (2008). Sensory deficit in parkinson's disease: Evidence of a cutaneous denervation. Brain, 131(7), 1903–1911. https://doi.org/10.1093/brain/awn102

O'Connor, D. H., Krubitzer, L., & Bensmaia, S. (2021). Of mice and monkeys: Somatosensory processing in two prominent animal models. *Progress in Neurobiology*, *201*, 102008. https://doi.org/10.1016/j.pneurobio.2021.102008

Palomar, F. J., Díaz-Corrales, F. J., Carrillo, F., Férnandez-Del-Olmo, M., Koch, G., & Mir, P. (2011). Sensory perception changes induced by transcranial magnetic stimulation over the primary somatosensory cortex in Parkinson's disease. Movement Disorders, 26(11), 2058–2064. https://doi.org/10.1002/mds.23779

Panayi, M. C., & Killcross, S. (2021). The role of the rodent lateral orbitofrontal cortex in simple Pavlovian Cue-Outcome learning depends on training experience. Cerebral Cortex Communications, 2(1). https://doi.org/10.1093/texcom/tgab010

Pei, Y.-C., Hsiao, S. S., Craig, J. C., & Bensmaia, S. J. (2010). Shape invariant coding of motion direction in somatosensory cortex. PLoS Biology, 8(2). https://doi.org/10.1371/journal.pbio.1000305

Petersen, C. C. (2019b). Sensorimotor processing in the rodent barrel cortex. Nature Reviews Neuroscience, 20(9), 533–546. https://doi.org/10.1038/s41583-019-0200-y

Pho GN, Goard MJ, Woodson J, Crawford B, Sur M. Task-dependent representations of stimulus and choice in mouse parietal cortex. Nat Commun. 2018 Jul 3;9(1):2596. doi: 10.1038/s41467-018-05012-y. Erratum in: Nat Commun. 2019 Jan 18;10(1):389. PMID: 29968709; PMCID: PMC6030204.

Rasmussen, Rune, and Keisuke Yonehara. 2020. "Contributions of Retinal Direction Selectivity to Central Visual Processing." *Current Biology* 30 (15): R897–903. https://doi.org/10.1016/j.cub.2020.06.002.

Rochefort, N. L., Narushima, M., Grienberger, C., Marandi, N., Hill, D., & Konnerth, A. (2011c). Development of direction selectivity in mouse cortical neurons. Neuron, 71(3), 425–432. https://doi.org/10.1016/j.neuron.2011.06.013

Sofroniew, N., Vlasov, Y. A., Hires, S. A., Freeman, J., & Svoboda, K. (2015). Neural coding in barrel cortex during whisker-guided locomotion. eLife, 4. https://doi.org/10.7554/elife.12559

Watanabe, H., Kojima, S., Otsuru, N., & Onishi, H. (2020). The repetitive mechanical tactile stimulus intervention effects depend on input methods. Frontiers in Neuroscience, 14. https://doi.org/10.3389/fnins.2020.00393

Whitsel, B L, James R. Roppolo, and Gerhard Werner. 1972. “Cortical Information Processing of Stimulus Motion on Primate Skin.” *Journal of Neurophysiology* 35 (5): 691–717. https://doi.org/10.1152/jn.1972.35.5.691.

Zainos, Antonio, Hugo Merchant, Antonio Hernandez, Emilio Salinas, and Ranulfo Romo. 1997. “Role of Primary Somatic Sensory Cortex in the Categorization of Tactile Stimuli: Effects of Lesions.” *Experimental Brain Research* 115 (2): 357–60. https://doi.org/10.1007/pl00005704.

Zhao, S., Ting, J. T., Atallah, H. E., Qiu, L., Tan, J., Gloss, B., Augustine, G. J., Deisseroth, K., Luo, M., Graybiel, A. M., & Feng, G. (2011). Cell type–specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function. *Nature Methods*, *8*(9), 745–752. https://doi.org/10.1038/nmeth.1668