# Movement-related activity surpasses touch responses in secondary somatosensory thalamus

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# Abstract

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Each primary sensory cortex gets input from corresponding primary and secondary thalamic nuclei. While primary thalamic nuclei are characterized by their sensory responses, the degree to which secondary thalamus encodes sensory and non-sensory signals is unknown. In the whisker system, the primary nucleus is the ventral posterior nucleus (VPM) and the secondary nucleus is the posterior medial nucleus (POm). While VPM sends precise whisker touch signals to cortex, POm responses are not well understood. Unlike VPM, POm is interconnected with many cortical areas, including motor cortex and association areas. POm, as a recipient of both bottom-up whisker signals and top-down cortical signals, might integrate touch with contextual signals such as reward or movement.

Using two-photon microscopy through a gradient index (GRIN) lens, I have assessed the POm response to touch with multi-whisker passive deflections of different velocities, to reward with water droplets, and to self-movement by measuring whisking and licking. POm activity had weak touch responses and was dominated by self-generated movements. My results suggest that POm is driven by self-movement or the internal state signals that accompany it, such as arousal.

Next, I investigated whether these representations change when mice learn sensory-reward associations. I demonstrate that POm activity continues to be dominated by whisking and licking and does not acquire selectivity for reward-associated sensory stimuli. We propose a model in which the representation of movements within POm may facilitate learning sensory features in cortex by creating a window for plasticity around relevant stimuli.

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# Chapter 1: Introduction

The sense of touch is an integral component of nearly every task an animal, including a human, must carry out. We use touch to manipulate and pick up objects, in our social communication (both in person and over text in the modern era), and even to help with eating. Touch is often used to actively explore an object, be it to search for a set of keys in a bag or to tap whiskers against an object. This active exploration allows animals to create a percept of the objects in the world and react appropriately. Although the anatomy of the touch system is well described, it remains unknown how the brain integrates touch input across the many sensors to create a sensory percept and representation of the external world. The creation of this representation is further complicated by the tendency of the animal to move the sensor itself as it explores.

Touch information about the face is processed in complex sensorimotor thalamocortical loops (Kleinfeld, Ahissar, and Diamond 2006). Primary somatosensory cortex (S1) receives information from two pathways through the thalamus: one through primary somatosensory thalamus (the ventral posterior medial nucleus, VPM) and one through secondary somatosensory thalamus (the posterior medial nucleus, POm). While primary thalamic nuclei like VPM are thought to be part of the main feedforward pathway for touch information and are characterized by their spatiotemporally precise sensory responses, whether secondary thalamic nuclei like POm provide feedforward or feedback information and the degree to which that information is sensory or non-sensory is unknown. Unlike VPM, POm is interconnected with many cortical areas, including motor cortices and higher order sensory areas (Aldes 1988; Bucci, Conley, and Gallagher 1999; Rubio-Garrido et al. 2009; Liao et al. 2010; Hooks et al. 2013; Olsen and Witter

2016). POm, as a recipient of both bottom-up touch signals and top-down cortical signals, might integrate touch with non-somatosensory signals such as reward or movement.

In this thesis, I aim to take steps closer to determining the role of POm in touch processing. I will first describe the neural circuitry and processing underlying touch, with a focus on primary and secondary thalamic nuclei. Subsequently, I will describe experiments performed to assess the contribution of touch and movement signals within POm. Finally, I will discuss activity of POm in the context of learning to associate a tactile stimulus with reward.

## 1.1 The whisker system of the rodent

To study touch, we turn to the somatosensory system of mice and rats. Whisker touch is an important sense mice and rats use to navigate and explore the world around them (Vincent 1912). These rodents scan the physical world by moving their whiskers back-and-forth in a motion called whisking (Welker 1964). Upon encountering an object, they will orient their heads towards the object and palpate the surface with multiple taps of their whiskers (Grant, Sperber, and Prescott 2012). Whiskers are able to discriminate size, shape, orientation, and texture to identify the objects in front of the animal (Guić-Robles, Valdivieso, and Guajardo 1989; Carvell and Simons 1990; Brecht, Preilowski, and Merzenich 1997; Krupa et al. 2001; Polley, Rickert, and Frostig 2005; Kim et al. 2020; Brown et al. 2021; Rodgers et al. 2021). Whiskers also aid in navigation and have been shown to track the animal's speed as it runs along the ground (Chorev, Preston-Ferrer, and Brecht 2016). Rats use this system for social communication as well, tapping their whiskers against the whiskers and face of conspecifics (Wolfe, Mende, and Brecht 2011).

Not only is the whisker system crucial to the experience of a rodent, but it also provides a great model for studying touch since it is anatomically and physiologically well-characterized

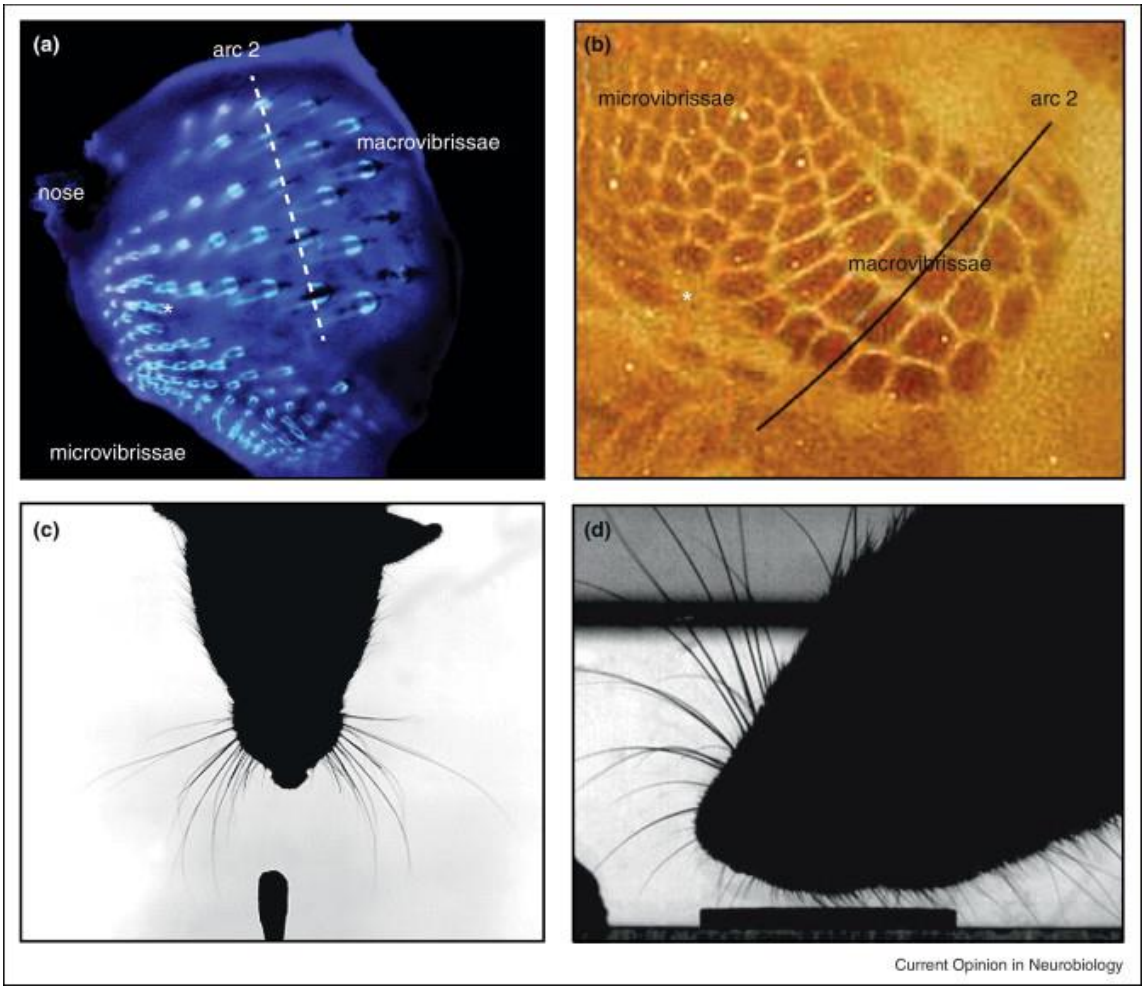



**Figure 1-1 Mystacial vibrissae of the rodent** (Deschênes, Moore, and Kleinfeld 2012)
A) Whisker follicles on the mystacial pad of the rat and near the mouth. "Arc 2" labels the second vertical column, or arc, of whiskers. B) Staining showing the barrels within S1. Arc 2 is also labeled here. C) Rat moving its macrovibrissae towards a swab with an odor. D) Rat investigating a coin with the microvibrissae.

and provides a convenient anatomical organization allowing for varying levels of modularity in studying the ascension of sensory signals to the cortex.

Mouse whiskers, or vibrissae, can be found on the face, including on the mystacial pad, or cheek. Most of these facial whiskers fall into either a category of macro- or microvibrissae. The microvibrissae are tiny whiskers focused around the mouse's mouth and nose (Figure 1-1D). These whiskers may be specialized for identifying small features of objects (Brecht, Preilowski,

and Merzenich 1997). The macrovibrissae are larger, more conspicuous whiskers on the sides of the face (Figure 1-1C) (Brecht, Preilowski, and Merzenich 1997). The majority of research in the whisker system describes the neural and behavioral aspects of the macrovibrissae, which have a prominent representation in the primary somatosensory cortex. These whiskers are organized into an orderly grid across each side of the face (Figure 1-1A) (Brecht, Preilowski, and Merzenich 1997). As somatosensory information ascends through the central nervous system, this arrangement of whiskers is conserved in the responses of clusters of cells at each stage. For each macrovibrissa, there is a corresponding barrelette in the brainstem, a barreloid in the thalamus, and a barrel in the primary somatosensory cortex (S1) (Figure 1-1B) (Ma 1991; Van Der Loos 1976; Woolsey and Van der Loos 1970). These barrelettes, barreloids, and barrels are mapped in space in the same order and location as the whiskers on the face, providing an easily identifiable anatomy.

Similar to many other sensory systems, whisker touch is an active behavior in which mice move their whiskers as they explore (Vincent 1912; Brecht, Preilowski, and Merzenich 1997). Thus, the whisker system provides a good model for studying the interplay of sensation and movement. Two sets of muscle groups within the mystacial pad, where the whiskers are located, control whisker movements: the intrinsic muscles that are anchored within the mystacial pad, and the extrinsic muscles that are anchored outside the mystacial pad (Dörfl 1982; Haidarliu et al. 2010). The intrinsic muscles form slings around the whisker follicles and their activation protracts, or brings forward, the whiskers (Berg and Kleinfeld 2003). The extrinsic muscles can shift the pivoting point of the whiskers and are involved in the retraction, or return to backward resting position (Berg and Kleinfeld 2003). Whisking, the back-and-forth movement of the whiskers, is a rhythmic behavior that has been well described. In whisking, the whiskers move in

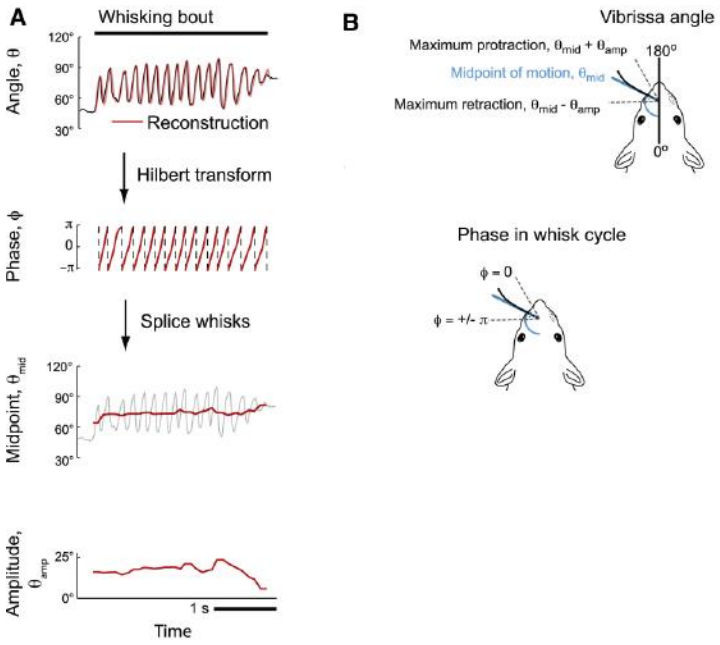



**Figure 1-2 Fast and slow components of whisking**
(adapted from Hill et al. 2011)
A) How components of whisking map onto whisking angle. (Midpoint is also known as set point.) B) Diagram of whisker angle and phase

a trajectory that is mostly rostral-caudal with a dorsal-ventral displacement and torsional rotation of each whisker within the follicle (Bermejo, Vyas, and Zeigler 2002; Knutsen, Biess, and Ahissar 2008). Since it is somewhat oscillatory, whisking can be described by both fast- and slow-varying timescales (Figure 1-2). The whiskers are described as moving around a set-point, the center of the oscillation. The phase, a fast component of whisking, describes the rostral-most and caudal-most tips of the oscillation around the set point. The amplitude, a slow component of whisking, describes the size of the envelope or amount of back-and-forth whisking, and is the distance between the rostral and caudal ends of the oscillation. The brain might control and represent these fast and slow signals of whisking separately. Many cells in primary motor cortex encode slow components of whisking like amplitude and set point (Hill et al. 2011). Neurons in S1 have been shown to encode both fast and slow components of phase, amplitude, and set point (Crochet and Petersen 2006; Curtis and Kleinfeld 2009; Rodgers et al. 2021).

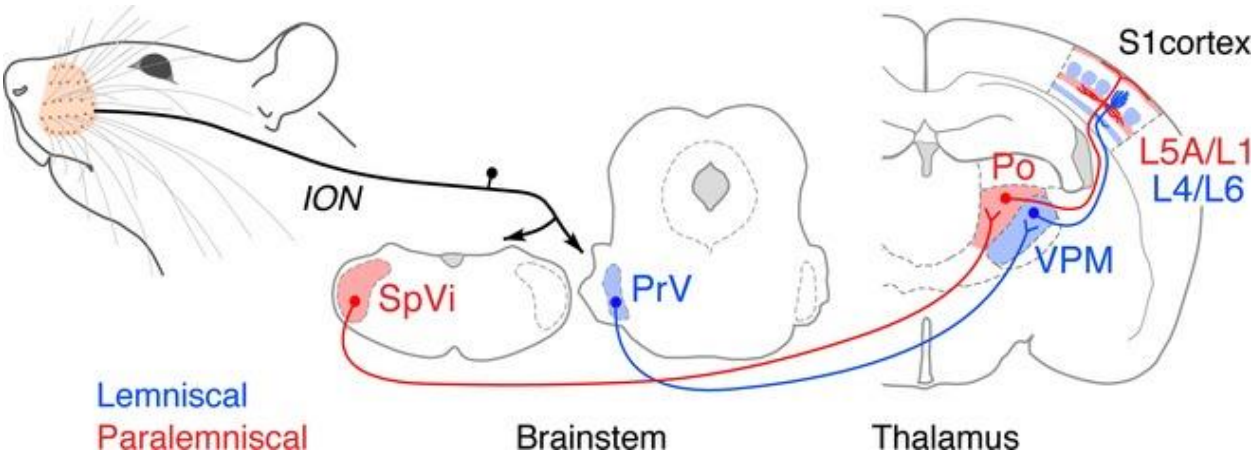



**Figure 1-3 Lemniscal and paralemniscal pathways** (Frangeul et al., 2014)
In the leminscal pathway (blue), sensory information from the whisker travels from the infraorbital nerve (ION), or trigeminal nerve, to the principal nucleus (PrV), then to the ventral posterior medial nucleus (VPM), and then to middle and deep layers (layers 4 and 6) of primary somatosensory cortex (S1). VPM is also known to project to layer 5b of S1, not shown. In the paralemniscal (red) pathway, sensory information from the trigeminal nerve travels to the interpolar division of the spinal trigeminal nucleus (SpVi), then to the posterior medial nucleus (POm, here labeled PO), and then to superficial and deep layers (layer 1 and layer 5a) of S1.

## 1.2 The lemniscal and paralemniscal pathways for ascending touch information

The ascending path of whisker information to the cortex can be divided into two main streams: the lemniscal and the paralemniscal. These parallel pathways to the cortex both follow a similar organization. Whisker information originates in the mechanoreceptors of the follicle and is detected by primary afferent sensory neurons in the trigeminal ganglion. These neurons respond to the touch of individual whiskers at short latencies and encode a number of parameters regarding whisker touch, including onset and offset, duration, direction, velocity, size of deflection (amplitude), and temporal pattern (Zucker and Welker 1969; Lichtenstein, Carvell, and Simons 1990). The trigeminal ganglion sends this information to trigeminal brainstem nuclei, where it passes to the thalamus, then to S1 (Figure 1-3). These pathways are theorized to have different functional roles due to their differing anatomical projections and functional

responses. The lemniscal pathway is known to reliably transmit sensory signals to S1 in a spatially and temporally precise way. The function of the paralemniscal pathway is less clear.

The lemniscal pathway is the canonical path through which somatosensory information reaches the cortex. This pathway is characterized by a strict topographical organization and fast, reliable responses to whisker touch. The lemniscal pathway begins with the principal nucleus (PrV) in the brainstem, which receives input from the infraorbital branch of the trigeminal nerve (Clarke and Bowsher 1962; Hayashi 1980). Groups of neurons in PrV form the barrelettes that match the whisker organization on the face (Ma 1991). Like neurons in the trigeminal ganglion, neurons in PrV tend to respond reliably and with short latencies to whisker stimulation and encode direction, amplitude, and velocity of whisker stimuli (Shipley 1974; Minnery and Simons 2003). They have larger receptive fields that respond primarily to the whisker corresponding to their barrelette location (the principal whisker), but to a lesser extent to one or more adjacent whiskers (Shipley 1974; Minnery and Simons 2003). PrV sends a large projection to VPM, through which neurons within a barrelette project to the corresponding barreloid in VPM (Ma 1991). VPM neurons respond the most and at a fast latency to a principal whisker but also respond to an average of 2-4 adjacent whiskers (Armstrong-James and Callahan 1991; Chiaia et al. 1991; Diamond, Armstrong-James, and Ebner 1992; Moore et al. 2015). Like PrV, VPM neurons are also sensitive to direction, amplitude, and velocity of whisker deflection; however, VPM response magnitudes are smaller and fewer VPM neurons respond to touch with sustained activity (Ito 1988; D J Simons and Carvell 1989; Pinto, Brumberg, and Simons 2000; Minnery, Bruno, and Simons 2003). VPM neurons form barreloids, which project to their corresponding barrel in layer 4 of S1 (Van Der Loos 1976; Lu and Lin 1993; Land, Buffer, and Yaskosky 1995; Wimmer et al. 2010). VPM also sends input to layers 5b and 6a of S1 (Oberlaender et al. 2012;

Constantinople and Bruno 2013). The projection from VPM to S1 is bidirectional: corticothalamic neurons in layer 6 send feedback projections to S1 (Bourassa, Pinault, and Deschênes 1995; Killackey and Sherman 2003). Activating these feedback projections from a barrel's cortical column enhances the whisker-evoked responses within the corresponding barreloid (Temereanca and Simons 2004). However, silencing S1 does not affect VPM responses (Diamond et al. 1992).

The paralemniscal pathway is characterized by reduced somatotopy and long latency responses. Here, input from the infraorbital branch of the trigeminal nerve reaches the interpolar division of the spinal trigeminal nucleus (SpVi) in the brainstem (Ma 1991). Unlike the trigeminal ganglion, neurons in SpVi are distinctly multi-whisker and have no clear principal whisker (Jacquin, Mooney, and Rhoades 1986). In other ways, SpVi is similar to PrV. SpVi neurons exhibit reliable onset, offset, and duration responses to whisker stimuli (Sosnik, Haidarliu, and Ahissar 2001). From SpVi, information is sent to the secondary somatosensory thalamus, POm (Veinante, Jacquin, and Deschênes 2000). POm neuronal responses are not as well defined as VPM responses. POm lacks barreloids, but likely has a loose topographical map (Fabri and Burton 1991). POm neurons respond at a long latency to an average of 5 whiskers, with no clear principal whisker, while VPM neurons, though multi-whisker in nature, do have a clear principal whisker defining the center of the receptive field (Diamond, Armstrong-James, and Ebner 1992; Moore et al. 2015). POm neurons increase in firing rate with increased whisker deflection amplitude (Gharaei et al. 2020). Whether POm neurons have direction or velocity sensitivity remains unknown. POm axons projecting to S1 avoid the barrels, and instead ascend through the cortex in the space in between the barrels, called the septa, branching out across cortical columns within layer 1 and layer 5a (Koralek, Jensen, and Killackey 1988;

Chmielowska, Carvell, and Simons 1989; Lu and Lin 1993; Ohno et al. 2012; Wimmer et al. 2010). Like VPM, POm also receives corticothalamic feedback from S1. Layer 5b cells project to POm (Bourassa, Pinault, and Deschênes 1995). It is thought that S1, not SpVi might be the main input to POm neurons, since silencing S1 causes POm to be silenced (Diamond et al. 1992). Input from S1 may even amplify incoming sensory signals from the brainstem (Groh et al. 2014).

S1 is the cortical recipient of the lemniscal and paralemniscal pathways. At this stage of ascending somatosensory input, there has been considerable transformation of the touch signal. Layer 4 excitatory cells are more tuned to direction than VPM cells, while fast-spiking interneurons in S1 are less tuned to direction than excitatory cortical cells or VPM cells (Bruno and Simons 2002). The degree of directional tuning in cortical cells decreases as they receive more converging thalamocortical input (Bruno and Simons 2002). VPM cells are only slightly sensitive to the velocity of whisker movement (Pinto, Brumberg, and Simons 2000). In contrast, layer 4 cells of S1, which receive VPM input, do increase their overall firing rates with increasing velocity (Pinto, Brumberg, and Simons 2000). Increasing velocity also increases spiking in cells across layers in S1, including POm-recipient layers 2/3 and 5 (Wilent and Contreras 2004). How POm might contribute to whisker feature encoding in S1 is unknown, since many aspects of feature encoding in POm are not well described. Recently, Gharaei et al. demonstrated that POm responses in anesthetized animals scale with increasing whisker deflection amplitudes. In this study, POm was shown to be the mediator through which superior colliculus activity could increase S1 responses to whisker amplitude (Gharaei et al. 2020). Since the superior colliculus has been implicated in orienting and attention (Goodale and Murison 1975; McHaffie and Stein 1982; Robinson, Bowman, and Kertzman 1995), these experiments

suggest an interesting role for the paralemniscal pathway in which POm might adjust S1 feature encoding when the animal is directing attention to particular sensory objects.

## 1.3 Parallel or hierarchical pathways through thalamus

The organization of ascending pathways through a primary and secondary thalamus exists not only in the somatosensory system, but also in the visual and auditory systems (Guillery 1995). Since we know primary thalamic nuclei to be responsible for transmitting reliable sensory signals to the cortex, why does there exist a second path? It has been proposed that these pathways are not parallel, but rather form a hierarchy of thalamic and cortical processing (Guillery 1995; Sherman and Guillery 2011). In this framework, nuclei can be described as either drivers or modulators based on the types of synapses they have with a downstream region. Driver synapses are formed by large axonal terminals and by activating ionotropic glutamate receptors are able to initiate larger postsynaptic responses (Sherman and Guillery 2011). Modulator synapses, on the other hand, are made by smaller terminals, activate metabotropic and ionotropic glutamate receptors, and thus initiate smaller postsynaptic responses (Sherman and Guillery 2011). By these definitions, whisker touch information ascends to VPM, which then drives S1 (Bruno and Sakmann 2006). S1 sends driving information to POm, which projects to middle layers of secondary somatosensory cortex (S2) with driving synapses (Groh et al. 2014; Viaene, Petrof, and Sherman 2011). The POm synapses onto S1 are classified as modulators, not drivers (Viaene, Petrof, and Sherman 2011). Thus, Murray Sherman classifies primary and secondary thalamus into first-order thalamic nuclei that precede cortical processing and higher-order thalamic nuclei that follow primary sensory cortex in the sensory hierarchy and facilitate corticothalamocortical information transmission. Sensory information is described as passing through a first-order thalamic nucleus (VPM) to primary sensory cortex (S1) then via

corticothalamocortical transmission through a higher-order nucleus (POm) to higher-order sensory cortex, or secondary somatosensory cortex (S2) (Theyel, Llano, and Sherman 2010; Sherman and Guillery 2011). In support of this organization, silencing S1 renders POm silent and silencing POm renders S2 silent (Diamond et al. 1992; Theyel, Llano, and Sherman 2010).

Adding to the idea that POm might be a higher-order nucleus, POm (but not VPM) is densely interconnected with many cortical regions. In addition to input from S1, POm also receives input from S2, primary motor cortex, and posterior parietal cortex (Aldes 1988; Bucci, Conley, and Gallagher 1999; Liao et al. 2010; Olsen and Witter 2016). Furthermore, POm projects to a diverse set of cortical areas including S2, primary motor cortex, primary and secondary visual cortex, and auditory areas (Rubio-Garrido et al. 2009). These data suggest that POm may not solely be involved in relaying somatosensory information to S1, but rather may have access to contextual information from across the brain.

## 1.4 Theories of the posterior medial thalamus

Despite decades of study, there remains no consensus on the role POm plays in whisker sensation. There have been many, often contradictory, ideas explaining the role of POm in the whisker system.

### *1.4.1 POm encodes multi-whisker touch*

Given that POm receives its main ascending input from the multi-whisker SpVi and that POm receptive fields are large and exhibit no clear principal whisker response, it is thought that POm neurons might be activated by multi-whisker touch. Indeed, in the anesthetized rat, Diamond et. al. reported that POm neurons were more strongly activated by stimulating the entire whisker pad than by deflecting individual whiskers (Diamond, Armstrong-James, and Ebner 1992). In fact, POm may be a center for multi-whisker input such that it is not involved in

single-whisker touch processing. POm has been shown to be unnecessary for performance in a single-whisker sensory discrimination task. Narumi et al., trained rats on a go/no-go task to discriminate direction of single whisker deflections. Lesions in POm did not affect the animals' ability to perform the task, while lesions in VPM, reduced performance (Narumi et al. 2007). These results also held true with muscimol injections into POm and VPM (Nakamura et al. 2009). Thus, POm is not necessary for discriminating between the forward and backward deflections of a single whisker. Nevertheless, further studies are needed to determine if POm is necessary for multi-whisker integration of touch signals.

### *1.4.2 POm encodes pain*

Low-threshold mechanosensation may not be the main function of POm. Frangeul et. al. proposed that POm may play a specialized role in pain processing. After experiencing back-and-forth single whisker deflections, VPM neurons exhibited expression of cFos, and immediate early gene that marks high levels of neural activity, but POm neurons did not (Frangeul et al. 2014). When mice were allowed free exploration of an enriched environment, cFos labelling was substantial in VPM but not POm (Frangeul et al. 2014). In contrast, injection of capsaicin into the whisker pad yielded strong cFos expression in POm, but not VPM (Frangeul et al. 2014). These experiments suggest that POm may be involved in a pain-specific pathway. Pain, however, is often a stimulus that invokes high arousal states. Given evidence of modulation of POm responses during high arousal states (see section 1.4.4 below), more studies are needed to determine if POm responses specifically to pain or if increased activity in POm is due to the high arousal caused by painful stimuli.

### *1.4.3 POm encodes whisker movement*

Some have theorized that POm may not be involved in touch processing at all. Yu et. al., classified cells in VPM and POm as whisking-related, touch-related, or mixed in anesthetized rats. They initiated fictive whisking with stimulation of the facial nerve in touch-free blocks and in touch blocks when a pole was moved through the "whisking" whiskers (Yu et al. 2006). POm cells had a touch index (the difference in responses during protraction in the touch and touch-free blocks divided by the sum) close to zero (Yu et al. 2006). This result suggests that POm neurons encode whisking movement and not touch. It was theorized that this movement signal, when combined with a touch signal from VPM, might allow the animal to recover the position in the whisker field where an object was touched.

However, the whisking signals found in POm are likely not fine-grained enough to allow for object localization. By monitoring self-generated whisking in awake animals while recording in thalamus, studies have shown that the majority of POm cells are not modulated by whisker phase (Moore et al. 2015; Urbain et al. 2015). Instead, most VPM cells are modulated by this fast component of whisking (Moore et al. 2015; Urbain et al. 2015). Our lab has similarly found that most POm neurons do not encode whisking phase, but rather are highly correlated with whisking amplitude (Petty et al. 2020).

Since the whisker system is not known to have proprioceptive input (Moore et al. 2015), whisking signals in somatosensory thalamus would either arise from reafference of sensory stimulation in the whisker follicle as the whiskers move through the air or from a motor efference copy sent from motor centers of the brain to thalamus. It is thought that whisking signals in VPM are due to sensory reafference (Moore et al. 2015). However, sensory reafference does not explain the POm correlation with whisking amplitude. Since ipsilateral whisking is

strongly coupled to contralateral whisking in air (Gao, Bermejo, and Zeigler 2001; Sachdev et al. 2003), paralyzing the mystacial pad contralateral to POm while monitoring ipsilateral whisking as a proxy for contralateral whisking can reveal whether whisking signals within POm are related to sensory reafference. After severing the buccal and upper marginal nerves (the motor nerves that control whisking) within the contralateral mystacial pad, POm activity continues to correlate with ipsilateral whisking, suggesting that the whisking signal within POm cannot be due to sensory reafference (Petty et al. 2020). Another possible explanation for the whisking signal within POm is that this signal is a motor efference copy, or a copy of the motor plan sent from motor regions to sensory regions of the brain. However, silencing primary motor cortex enhances, rather than depresses, the correlation of POm with whisking amplitude (Petty et al. 2020). This result suggests that the whisking amplitude-related signal seen in POm is not a motor efference copy sent from primary motor cortex.

### *1.4.4 POm is modulated by arousal and internal state*

The whisking amplitude signal seen in POm responses is also correlated with pupil dilation (Petty et al. 2020). This result suggests that the correlation of POm with whisking amplitude might be related to brain-wide arousal states. Indeed, neurons in secondary visual thalamus (the lateral posterior thalamus, or pulvinar) also exhibited correlation with whisking amplitude and pupil dilation (Petty et al. 2020).

If POm is following arousal-based brain states rather than sensory or motor aspects of whisking amplitude, what affect does arousal have on POm's role in the whisker system? POm neurons in anesthetized rats were more likely to respond to air puffs to the whiskers when brainstem cholinergic nuclei were stimulated (Masri et al. 2006). Brainstem cholinergic systems are involved in transitions from sleep to alert in the thalamus (Steriade 2003). POm responses to

multi-whisker deflection were also more functionally connected, as measured by cross-trial correlation of local field potentials (Sobolewski et al. 2010), to S1 responses when rats were in high arousal states (Sobolewski et al. 2015). These data suggest that modulation of POm by arousal might increase POm responses to whisker touch. Studies of POm responses to whisker touch at the single cell level while arousal states are modulated are needed to determine if this hypothesis is correct.

### *1.4.5 POm involvement in cortical plasticity and learning*

It is possible that POm plays a role in sensory learning and cortical plasticity. Gambino et. al. found that a rhythmic whisker stimulation protocol could induce long-term potentiation (LTP) in layer 2/3 neurons of S1 of anesthetized mice. This LTP was mediated by NMDAR-dependent subthreshold depolarization plateaus (Gambino et al. 2014). By optogenetically activating POm terminals in layer 1, they were able to induce these NMDAR-dependent plateaus in L2/3 neurons (Gambino et al. 2014). Furthermore, when silencing POm with muscimol, the whisker stimulation protocol did not induce LTP in S1 neurons (Gambino et al. 2014). These results suggest that POm activity may support the potentiation of sensory signals in S1. Indeed, work done *ex vivo* suggests that POm may disinhibit layer 2/3 apical dendrites, facilitating plasticity of incoming sensory inputs (L. E. Williams and Holtmaat 2019). Furthermore, POm, but not VPM, is capable of persistent depolarization, which could promote plasticity (Zhang and Bruno 2019).

Not only might POm facilitate plasticity in the cortex via the apical dendrites, it may also serve to increase the gain of sensory responses as they happen. Since POm densely innervates layer 1 of S1, where it can influence apical dendrites, it has been proposed that coincident activity of POm and ascending sensory input from VPM would serve to enhance sensory

responses (M. E. Larkum, Senn, and Lüscher 2004; M. Larkum 2013). Activating POm axons in S1 while stimulating the whiskers increased the probability of spiking in layer 5B cells of S1 even though activating POm alone did not increase probability of spiking (Mease, Metz, and Groh 2016). Activating POm axons in S1 also facilitated the response to whisker stimulation in layer 2/3 (Zhang and Bruno 2019).

The fact that POm activity can facilitate plasticity in the cortex and may enhance whisker sensory responses in cortical cells puts POm in a prime position to facilitate learning about sensory stimuli, particularly in cortical representations of those stimuli. Audette et. al. investigated the role of learning by examining the POm synapses to S1 in mice who had learned sensory-reward associations. After training to associate an air puff with rewards, *ex vivo* stimulation of POm terminals drove increased activity in layer 5, and subsequently in layer 2, of S1 (Audette et al. 2019). These effects were not seen in VPM synapses on S1 or in mice who received similar air puff experience without rewards (Audette et al. 2019). These data make clear that changes are happening in the POm-S1 thalamocortical circuit during learning. How POm is activated in learning sensory-reward associations remains unknown. In the Audette et. al. sensory-reward association paradigm, is POm responding to the sensory stimulus of the air puff, the whisking movements of the animal, or an arousal state of the animal induced by rewards? Studies of what POm activity represents during such a behavior and how these representations change while mice learn the association are needed.

## 1.5 Studies of POm activity in behaving mice

Since there are so many disparate ideas about POm's role in the whisker system, what then truly drives POm neurons? The majority of studies of POm function have been carried out in anesthetized animals. While anesthesia offers precise control of stimuli and fictive whisker

movements, it can also alter the impact of sensory stimuli and does not allow measurements of the effects of naturalistic whisker movement or awake brain states (Daniel J. Simons et al. 1992). Given the evidence that POm is impacted by arousal states and neuromodulation, it is essential to add to the body of research studies of POm in the awake animal. Furthermore, given POm's potential role in learning, studies of learned behaviors are particularly intriguing. While the number of studies of POm in awake animals is increasing, there have been relatively few studies of POm activity while mice are engaged in a behavioral task.

To our knowledge there have been two studies of POm activity recorded while animals are engaged in a behavioral task. In the first study, El-Boustani et. al. trained mice to lick to receive rewards when the C2 whisker was deflected and to withhold from licking when the B2 whisker was deflected. Calcium imaging of thalamic axonal boutons in S1 and secondary somatosensory cortex (S2) revealed that POm axons in S1 and S2 were more active on trials when the mouse licked than when the mouse did not lick (El-Boustani et al. 2020). VPM axons in S1 were also more active on trials when the mouse licked (El-Boustani et al. 2020). Since mice were often whisking when licking, El-Boustani et. al. hypothesized that the increase in VPM activity on lick trials was due to reafference from whisking. A whisker selectivity index calculated from responses to C2 and B2 deflections, revealed that VPM was less selective on lick trials (mice in this paradigm continued licking in response to no-lick B2 deflections about 50% of the time) (El-Boustani et al. 2020). POm axons in S1 were also less whisker selective on lick trials, yet axons in S2 from an anterior subregion of POm remained selective on lick trials (El-Boustani et al. 2020). These data suggest a new scenario in which POm sends topographic whisker-specific information to S2 but not to S1. Perhaps the S1-projecting POm cells are truly

modulators that inherit broad, multi-whisker information from S1 and the S2-projection POm cells form a parallel sensory stream of SpVi to POm to S2 that duplicates the lemniscal system.

In the second behavioral study, LaTerra et. al. trained mice to lick for rewards when a vibration stimulus was detected on the forepaw. Calcium imaging in POm axons in the forepaw region of S1 revealed that POm was more active during the response period on correct detection trials (LaTerra et al. 2020). The response period was when mice were licking more, so the increased activity in POm seen here is similar to that observed by El-Boustani et al., although less likely to be whisking-related given that these axons are more likely from forepaw regions of POm than whisker regions. Interestingly, in an alternate version of the task in which the mice were trained to withhold licking as their response to the forepaw vibration, POm remained most active during the "no-response" period (LaTerra et al. 2020). This data suggests that POm activity may not be motor-related. This study raises the possibility that POm may be signaling reward expectation. It is also possible POm may be more active due to arousal effects that likely also occur during times of reward expectation. The mice may also be whisking a lot during the response period or moving their limbs or body more. Regardless of whether POm is signaling something reward-related or arousal-related, the higher activity during the response period may be what contributes to plasticity effects described earlier. Higher activity in POm during the response period may facilitate the cortical association between the sensory stimulus and the reward.

Both of these studies involved calcium imaging of POm axonal bouton activity in S1. Axonal tracing of POm neurons has suggested that some POm neurons (1 out of 7 traced neurons) do not project to S1 at all (Ohno et al. 2012). Studies of POm somata would

complement this work well and allow for a more complete understanding of what POm activity encodes during whisker behavior and which of those signals is sent to S1.

An additional study included silicon probe recordings of neurons from POm in a visual contrast discrimination task assessing brain-wide responses (Steinmetz et al. 2019). Steinmetz et al. used a kernel-regression model to determine whether model fits to neurons were improved with specific predictors were included in the model. Approximately 5% of POm neurons were better predicted when choice-related parameters were included and approximately 10% of POm neurons were better predicted when action-related (moving a choice wheel to the left or right with the forepaws) parameters were included (Steinmetz et al. 2019). Since this study focused on finding brain-wide trends in behavior-related activity, there is not enough information about POm-relevant behavior, such as when the mice were whisking during the task, to make clearer statements about POm's role in this visuo-motor task.

## 1.6 Gradient index lenses provide a new way to study POm

Over the last several decades, studies of POm activity have often been carried out with electrophysiological techniques, often single unit electrodes or juxtasomal pipette recordings (Chiaia et al. 1991; Diamond, Armstrong-James, and Ebner 1992; Moore et al. 2015; Urbain et al. 2015). These techniques offer precise information about the timing of action potentials, but only offer data from a single neuron at once. Recent advances in silicon probes allow for simultaneous recordings in large numbers of neurons (Jun et al. 2017); however, these and other multiple single-unit tools have not yet been frequently used in the somatosensory thalamus (Waiblinger, Brugger, and Schwarz 2015; Steinmetz et al. 2019).

Calcium imaging with two-photon microscopy offers the ability to record activity from hundreds of neurons simultaneously in awake animals (Stosiek et al. 2003; Kerr et al. 2007;

Dombeck et al. 2007). Without additional technology, this technique is limited to depths of a few hundred microns from the brain surface due to the light-scattering properties of brain tissue (Oheim et al. 2001). POm, at a depth of approximately 3000 microns, is unreachable. Gradient index (GRIN) lenses have offered a solution to this challenge. These lenses bend light such that the image of deep tissue is mirrored at the surface (Levene et al. 2004). They are small enough in diameter (≤ 1000um) to implant in the brain yet still allow for single cell resolution (Jung et al. 2004; Levene et al. 2004).

Microendoscopes using GRIN lenses were recently used to investigate activity in VPM in awake mice during states of quiescence, whisking, and locomotion (Antonini et al. 2020). Since the axons from somatosensory thalamus travel laterally and then dorsally to reach the S1 barrel field, which additionally is located lateral to VPM, the implantation of the GRIN lens above VPM did not interfere with thalamocortical or corticothalamic connectivity (Antonini et al. 2020). Fluorescence transients in VPM were both more frequent and higher in amplitude in states of combined whisking and running (mice did not run without whisking) relative to during quiescence (Antonini et al. 2020). These results are in agreement with electrophysiology studies showing that VPM neurons have increased firing rates during whisking (Urbain et al. 2015; Moore et al. 2015). Non-negative matrix factorization analysis revealed that VPM neurons could be grouped into several sub-networks that had differing amounts of modulation by whisking (Antonini et al. 2020). As expected, most of these networks were positively modulated by whisking; however, some of the networks were suppressed by whisking (Antonini et al. 2020). These results would have been challenging to achieve without population analysis made possible by simultaneous recording of many neurons.

Calcium imaging through GRIN lenses also allows for careful investigation of spatial organization of signals among neurons. Antonini et al. discovered that the whisking modulated sub-networks within VPM were widely distributed, with groups of neurons in a network spanning distances of hundreds of microns. Studies have proposed that POm may have two subnuclei with different functions (Ohno et al. 2012; El-Boustani et al. 2020). The division between these subnuclei lies along the anterior-posterior plane (Ohno et al. 2012; El-Boustani et al. 2020). Imaging POm through a GRIN lens would provide an opportunity to further investigate the spatial organization of POm responses and the functional properties of neurons in these two subnuclei.

## 1.7 Overview

In this dissertation, I address remaining questions of POm function in the behaving mouse. In the next chapter, we determine how much of POm activity is due to sensory, motor, or reward-related signals. We use two-photon microscopy through GRIN lenses to measure POm activity in awake mice while delivering multi-whisker deflections at different velocities, supplying water rewards, and monitoring self-generated whisking and licking. Our experiments show that whisker movement best explains POm activity. In Chapter 3, we test whether POm representations change when mice learn sensory-reward associations. We demonstrate that movement signals dominate POm activity throughout learning. Overall, our results show that POm is strongly related to whisker movement, not touch. Our data, in combination with the reviewed experiments described above, support a model in which mice whisk when presented with salient, rewarded stimuli, which prompts the whisking-related response in POm to facilitate cortical learning about these stimuli.

# Chapter 2: Movement-related activity surpasses touch responses in secondary somatosensory thalamus

*Thank you to Gabriella Sahyoun for her essential contributions in histological processing and GRIN lens localization analysis to the following work, both in this chapter and in Chapter 3. As will become evident below, these pieces were key to our understanding of the data.*

## 2.1 Introduction

Sensing and perceiving the external world are processes of active exploration, in which sensory and motor systems are intricately intertwined (Gibson 1962). To scan the environment and objects around them, mice move their whiskers in a back-and-forth motion called whisking (Vincent 1912; Carvell and Simons 1990; Brecht, Preilowski, and Merzenich 1997). Somatosensory information from whisker taps against surfaces ascends to primary somatosensory cortex (S1) through the lemniscal pathway via the brainstem and a primary thalamic nucleus the ventral posterior medial nucleus (VPM) (Ma 1991; Lu and Lin 1993). S1 is also reciprocally connected with a secondary thalamic nucleus (often referred to as higher-order), the posterior medial nucleus POm (Veinante, Jacquin, and Deschênes 2000; Bourassa, Pinault, and Deschênes 1995). This nucleus also receives ascending information from the brainstem, but unlike VPM, is densely interconnected with many cortical regions, including secondary somatosensory cortex, primary motor cortex (M1), and association cortices such as posterior parietal cortex (Aldes 1988; Bucci, Conley, and Gallagher 1999; Rubio-Garrido et al. 2009; Liao et al. 2010; Hooks et al. 2013; Olsen and Witter 2016).

While primary thalamic nuclei like VPM are characterized by their spatiotemporally precise sensory responses (Armstrong-James and Callahan 1991; Chiaia et al. 1991; Diamond,

Armstrong-James, and Ebner 1992; Moore et al. 2015), the degree to which secondary thalamic nuclei like POm encode sensory and non-sensory signals is not well-understood. POm neurons have broad, multi-whisker receptive fields that are thought to respond best to multi-whisker stimuli (Diamond, Armstrong-James, and Ebner 1992). While regions that project to POm, such as the interpolar division of the spinal trigeminal complex (SpVi) in the brainstem, S1, S2, and the superior colliculus, are selective to whisker deflection direction (Roger and Cadusseau 1984; Bourassa, Pinault, and Deschênes 1995; Bruno and Simons 2002; Wilent and Contreras 2005; Furuta, Nakamura, and Deschenes 2006; Liao and Yen 2008; Hemelt et al. 2010; Kaloti et al. 2016), whether POm retains these sensory features is unknown.

Signatures of whisking movements have been reported in POm in both awake and anesthetized animals (Yu et al. 2006; Petty et al. 2020). However, only VPM shows activity modulated by fast parameters of whisking such as phase (Moore et al. 2015; Petty et al. 2020). Furthermore, whisking-related activity in POm is not due to sensory reafference of the whisker movements (Petty et al. 2020). When comparing active, non-whisking states with whisking states, which yield an increase in VPM firing rates, POm firing rates do not change (Urbain et al. 2015). In addition to whisking, POm firing rates are correlated with pupil dilation, a behavioral measure of arousal (Aston-Jones and Cohen 2005; Eldar, Cohen, and Niv 2013; Reimer et al. 2016; Petty et al. 2020), and POm has been theorized to be modulated by behavioral state (Masri et al. 2006; Trageser et al. 2006). POm axons in S1 have been shown to be active when mice lick for rewards in behavioral tasks (El-Boustani et al. 2020; LaTerra et al. 2020). Such rewarded stimuli can increase pupil diameter and arousal (Rudebeck et al. 2014). The extent to which POm might encode whisking movements versus rewards and behavioral state remains to be determined.

POm, as a recipient of both bottom-up touch signals and top-down cortical signals, might integrate touch with these non-somatosensory signals such as reward or movement. By imaging POm neurons through gradient index (GRIN) lenses, we recorded POm responses to touch, movement, and rewards in awake, head-fixed mice. Our data show that while some POm neurons may have velocity and direction selectivity, the majority of activity in POm is best explained by movements such as whisking and licking. Rewards did not modulate POm activity. The whisking-related signal in POm may represent either body movements or internal states that encourage movements, such as arousal and behavioral engagement.

## 2.2 Results

### *2.2.1 Assessing stimulus responses from a large population of thalamic neurons*

We first set out to compare POm responses to sensory stimuli, movement, and water rewards, a commonly used positive reinforcer, in the awake animal. Awake, head-fixed mice were presented with a vertical pole that moved horizontally through the whisker field, deflecting the majority of whiskers on the face (Figure 2-1A). To assess whisker deflection direction preferences, each touch trial involved a caudal-to-rostral sweep through the whisker field followed by a rostral-to-caudal sweep. To assess speed preferences, the pole moved at one of three different, randomly selected speeds on each touch trial (see 2.4.4 Experimental Design). A water droplet was delivered randomly as a fourth trial type. Since mice were water restricted, this water served as a reward that might manipulate arousal and behavioral state throughout the

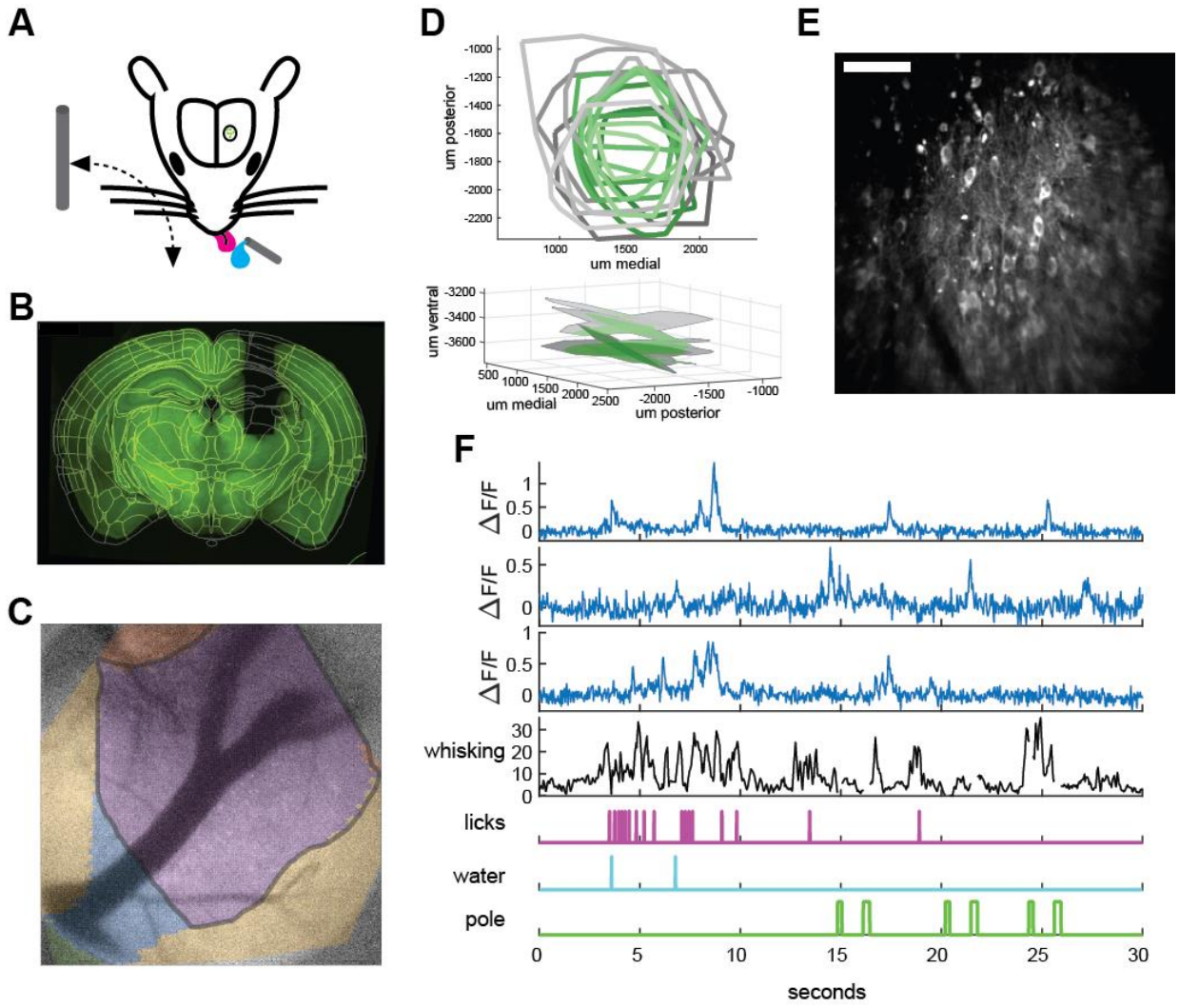



**Figure 2-1 Two-photon calcium imaging of POm neurons through GRIN lenses**
A) Experimental setup. Calcium imaging of GCaMP6F in POm neurons through a GRIN lens in awake mice. Whisker deflections delivered at three different speeds with a caudal-to-rostral and a rostral-to-caudal deflection on each trial. Water rewards were delivered randomly. B) Coronal brain slice fit to the Allen Brain Atlas showing the placement of the lens. C) Reconstruction of POm boundaries (purple) overlaid on a one photon image through the lens. D) Field of view locations (gray) relative to bregma across all 9 mice with boundaries of visible POm in green. Top, view in the anterior-posterior and medial-lateral plane. Bottom, view in 3D space. E) Two-photon image of thalamic neurons. Scale bar, 100um F) Example activity traces of POm neurons along with contralateral whisking amplitude, individual licks, water delivery, and pole whisker deflections.

experiment. To measure responses to facial movements, we monitored self-generated whisking and licking with video and capacitive touch sensors, respectively.

To survey as much as POm as possible, we sought to record many cells simultaneously. We injected a virus to deliver the gene for GCaMP6f into POm and implanted GRIN lenses above. Calcium activity was recorded from thalamic cells (Figure 2-1E) with two-photon microscopy, recording a range of 31 to 131 cells (mean 65.2) from each mouse (n = 9). The nuclear location of thalamic cells was identified by generating a map of the field-of-view through the lens (Figure 2-1C). To do this, we used the SHARP-Track program (Shamash et al. 2018) to align histological brain slices to the Allen Brain Atlas (Allen Institute for Brain Science 2015) and reconstruct the position of the GRIN lens in the brain (Figure 2-1B). Next, we located the plane lying at the working distance from the bottom edge of the lens and determined which nuclei were visible in this plane (Figure 2-1D,E). Our dataset included 10 to 73 neurons that we could confidently classify as POm (mean 44.6) in each of the mice (401 POm neurons out of 587 total imaged neurons). We then compared calcium activity with sensory, motor, and reward events (Figure 2-1F).

### *2.2.2 POm exhibits weak touch preferences*

POm exhibited weak responses to the touch stimuli (Figure 2-2A,B). 37% of POm neurons were touch responsive (150 of 401 neurons, Wilcoxon sign rank test, $p < 0.05$) while 44% were responsive to the water reward (177 of 401 neurons, Wilcoxon sign rank test, $p < 0.05$). 53% of POm neurons had below baseline responses to one or more type of touch trial (214 of 401 neurons) while 26% had below baseline responses to water reward (104 of 401 neurons). Surprisingly, the water reward also caused a higher change in fluorescence in POm than the touch trials (Wilcoxon sign rank test, $p < 10^{-8}$) (Figure 2-2B). Although the population responded less to touch than to water rewards, 22% of POm neurons had a preference for fast or slow whisker deflections (Figure 2-2C; 88 of 401, Wilcoxon rank sum test, $p < 0.05$) and 21%

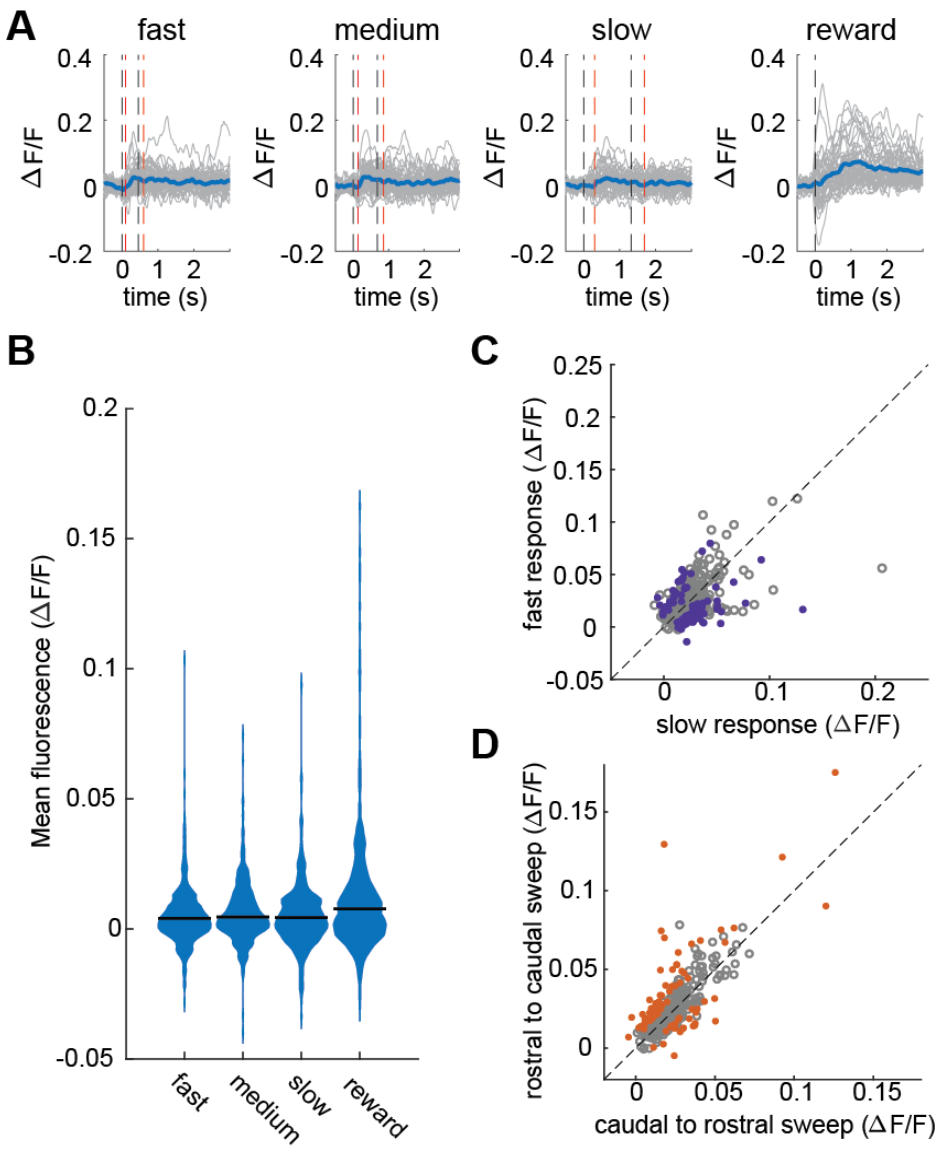



**Figure 2-2 POm exhibits weak touch preferences**

A) Event-triggered averages of POm calcium activity for an example mouse (n = 48 cells). Gray, individual neurons; blue, mean. Dotted lines indicate when pole deflection enters (black) and leaves (red) the whisker field. In right-most panel, dotted line indicates onset of reward consumption. B) Average fluorescence across a 2s window for each trial type (n=401 neurons). Black, medians. C) Average POm responses to fast and slow pole deflections (n = 401 neurons). Cells with significant preference in purple (22%). Population preference for speed is not significant (Wilcoxon rank sum test, p = 0.45. D) Average POm responses to caudal and rostral deflections (n = 401 neurons). Cells with significant preference in orange (21%). The POm population prefers rostral deflections (Wilcoxon signed rank test, $p<10^{-4}$).

exhibited preference for caudal or rostral deflections (Figure 2-2D; 86 of 401, Wilcoxon signed rank test, $p < 0.05$). 5% of POm neurons had preference for both speed and direction (19 of 401

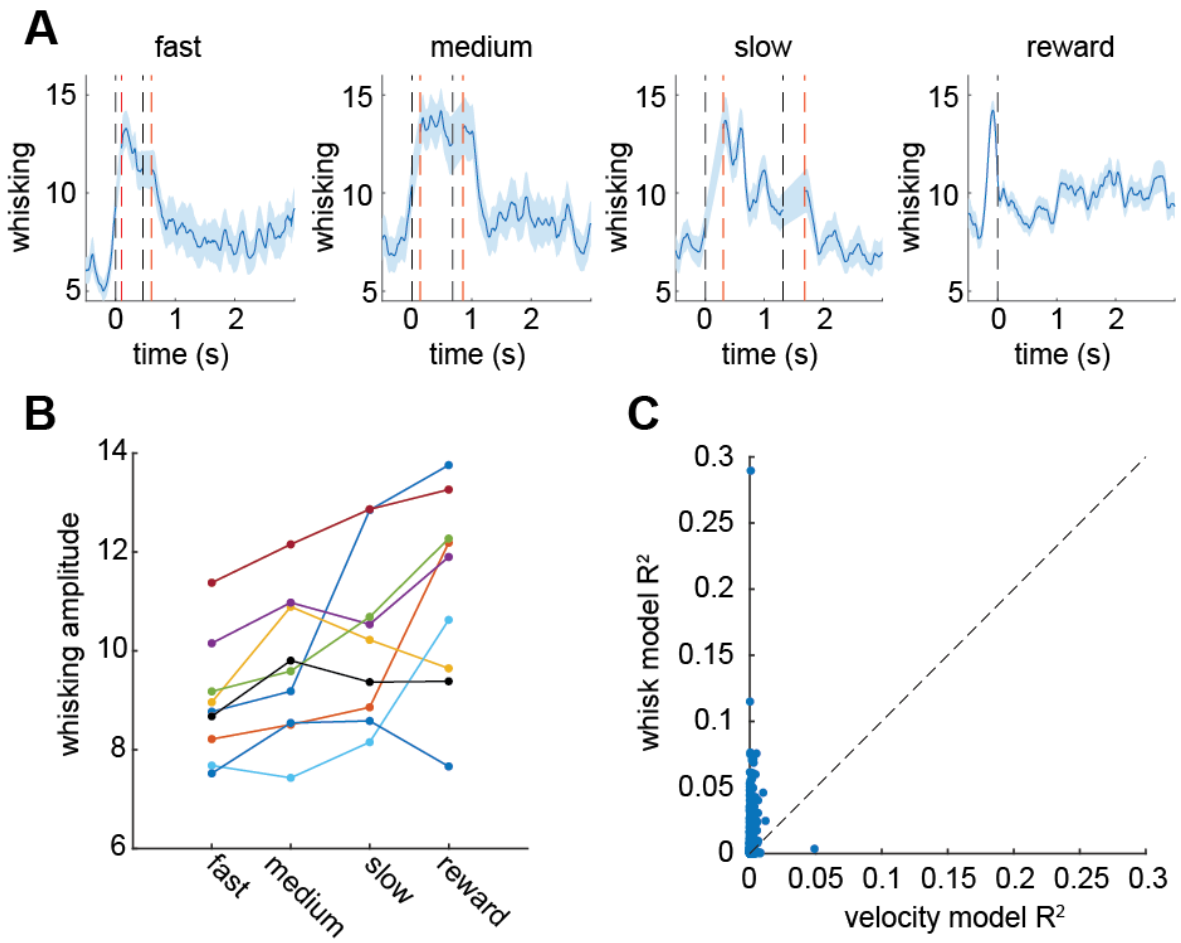



**Figure 2-3 POm activity on touch trials is better explained by whisking**
A) Event-triggered averages of contralateral whisking amplitude (shaded portion, standard error of the mean) for the example mouse in Figure 2-2A. Dotted lines indicate when pole deflection enters (black) and leaves (red) the whisker field. In right-most panel, dotted line indicates onset of reward consumption. B) Average contralateral whisking amplitude for each trial type. Color indicates mouse (n = 9). C) Model fits for whisking-only model and pole velocity-only model for each POm cell. n = 401 neurons from 9 mice.

neurons, Wilcoxon rank sum test, $p < 0.05$ for both speed and direction). Overall, the population did not show a significant speed preference (Wilcoxon rank sum test, $p = 0.45$), but did have a slight bias for rostral-to-caudal deflections (Wilxocon signed rank test, $p < 10^{-4}$).

### *2.2.3 Whisker movement best explains POm responses*

Mice do not sit passively while experimenters move poles through their whisker field. Video tracking of whisking contralateral to imaged POm revealed that mice tend to move their

whiskers during the pole deflection trials (Figure 2-3A,B). Furthermore, mice whisked more on the water reward trial than on the touch trials (Wilcoxon rank sum test, $p = 0.03$) (Figure 2-3B), which may explain why POm activity was greater on average for water rewards.

Given that POm activity was higher on reward trials, when mice were whisking more and that touch trials also include whisking, we wanted to know whether POm activity would be better explained by whisking or by whisker touch. To get at this question, we fit two bivariate linear regression models to each neuron. We modeled POm activity using touch velocity alone and using contralateral whisking amplitude alone. Comparing the variance explained by each model ($R^2$) revealed that whisking better explains activity in nearly every cell than velocity does (Figure 2-3E).

Since the pole deflects the contralateral whiskers, whisking cannot be measured reliably during the time when the pole is within reach of the whiskers (from black dashed line to red dashed line) but can be reliably measured between deflections (from first red dashed line to second black dashed line). In the aforementioned analyses, we interpolated across the pole deflection period. To measure whisking during the pole deflections, we also took video of the ipsilateral whiskers, which are not obstructed by the stimulus pole, in a subset of mice ($n = 5$). Whisking is often bilaterally synchronous (Gao, Bermejo, and Zeigler 2001; Sachdev et al. 2003), so we used ipsilateral whisking during the deflection period as a proxy for contralateral whisking. Analysis of whisking motion, or average amount of pixel intensity change in the whisker area of the video, revealed that 4 out of 5 mice had significantly more ipsilateral whisking on fast trials (Wilcoxon rank sum tests, $p < 10^{-3}$ for each 4 mice) and 3 out of 5 mice had more ipsilateral whisking during caudal-to-rostral deflections (Wilcoxon signed rank tests, p

$< 10^{-3}$ for each 3 mice) (Supplemental Figure 2-5). These results do not explain the slight preferences the POm population had for rostral-to-caudal deflections.

Because our mice are awake, many signals that might affect POm are overlapping. When we deliver touch stimuli, the mice whisk. When we deliver rewards, the mice lick and sometimes whisk. Which of these signals affect POm activity? To disentangle these correlated variables, we fit a multiple linear regression model to each POm neuron attempting to explain its activity with whisking, touch velocity, licking, and rewards. These models accounted for 0-29% of the variability in fluorescence activity (Figure 2-4A), which is typical for these types of analyses of stochastic processes (Ramirez et al. 2014; Peron et al. 2015; Musall et al. 2019). To determine the contribution of each predictor to POm activity, we modeled POm activity with that predictor

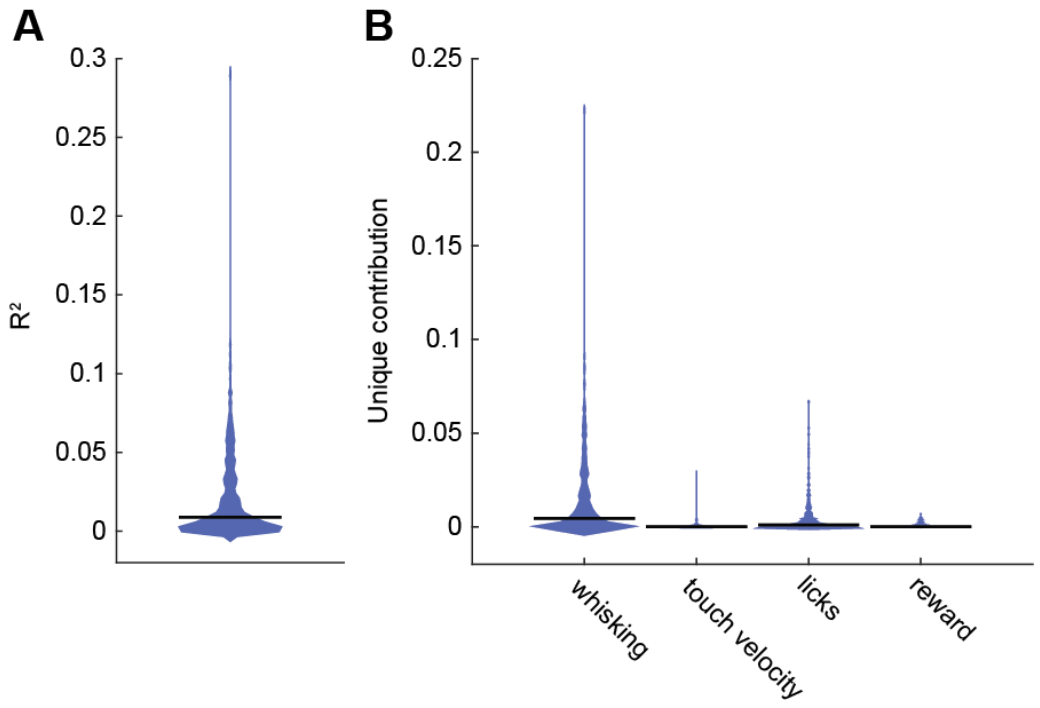



**Figure 2-4 POm activity is most strongly modulated by whisking**
A) Proportion of variance explained (adjusted $R^2$) by multiple linear regression for each cell (n = 401 neurons from 9 mice). Black line shows median $R^2$ value of the population. B) Unique contributions of each regressor in the model for each cell. Calculated from the $R^2$ of the cell's model with that regressor shuffled subtracted from the $R^2$ for that cell. Black, medians.

shuffled in time and then calculated its unique contribution as the difference in $R^2$ of the full model and the model with the shuffled predictor, similar to analyses applied to mouse cortex (Musall et al. 2019). This analysis revealed that whisking correlates with POm activity far more than touch, licking, or reward does (Figure 2-4B). Licking was a far second, contributing to the activity of some POm neurons (Figure 2-4B), which may suggest that POm is responsive to either body movements in general or to a brain state that encourages whisking and licking, such as general arousal or behavioral engagement.

## 2.3 Discussion

Our experiments show that POm activity is best explained by whisking. In the awake mouse, POm neurons do have a mild touch response to high velocity, multi-whisker pole deflections, but these responses are mostly explained by the concurrent whisking movements mice have in reaction to these deflections. These data are consistent with measurement of touch responses in POm in anesthetized and awake rats (Diamond, Armstrong-James, and Ebner 1992; Yu et al. 2006; Moore et al. 2015). POm had been reported to respond more to multi-whisker touch (Diamond, Armstrong-James, and Ebner 1992), but multi-whisker air puff stimulation had been shown to activate POm less than VPM in awake rats (Moore et al. 2015). Sensory responsivity and motor modulation had not previously been directly compared with one another in POm. Our data disentangles the concurrent touch and whisking responses that can happen in awake animals and clarifies that the majority of POm activity is due to whisking, not touch.

The finding that POm is more related to whisking than touch is consistent with previous observations of whisking-related activity in POm. POm activity has been shown to be correlated with whisking amplitude, but is not modulated by fast components of whisking like phase (Moore et al. 2015; Petty et al. 2020). In an anesthetized preparation involving air puffs and

experimenter-induced fictive whisking in rats, POm was shown to respond more to whisking than to touch (Yu et al. 2006). Electrical stimulation in fictive whisking is difficult to interpret because it can activate a variety of fiber types, including those associated with pain, which POm has also been speculated to mediate. Our study demonstrates that POm activity covaries with natural whisking in awake mice independent of activation of the pain system.

Even though whisking contributed most to POm responses, the POm population had a slight bias towards rostral deflections. One detail that complicates this result is that rostral deflections are always preceded by caudal deflections in our experiment. Since the order of directional deflections were not random, mice could likely predict the rostral deflections. The fact that POm is biased towards the predicted deflection might reflect less of a touch preference but rather support the hypothesis that POm is modulated by internal state. However, we cannot rule out that whisking was the culprit for these preferences. We used ipsilateral whisking, which is often synchronous with contralateral whisking (Gao, Bermejo, and Zeigler 2001; Sachdev et al. 2003), as a proxy for contralateral whisking, which is not measurable during the pole deflections. This control revealed that 3 of 5 mice whisked more during caudal deflections than during rostral deflections, but that 2 of the mice did not have significant differences in whisking for the different directions. A caveat of this control is that bilateral whisking can become asynchronous upon object contact (Carvell and Simons 1990; Mitchinson et al. 2007; Sachdev et al. 2003). When rats contact an object, bilateral whisking becomes asynchronous such that the whisking on the contacted side increases in amplitude (Sachdev et al. 2003). Thus, it is possible that our ipsilateral control, which is a measure of whisking amplitude on the non-contacted side, is underreporting whisking. Since the rostral deflection always follows the caudal deflection, the rostral deflection may be more susceptible to this underreporting. Given the results of the

regressions, in which whisking dominates POm activity, we believe that the bias towards rostral deflections seen in POm is likely an effect of whisking.

Although stimuli were presented in the dark at randomized inter-trial intervals, it is possible the mice were able to anticipate the presence of pole deflections and water rewards. The motor that controlled the pole deflections begins moving the pole closer to the whiskers prior to the touch onset window when the pole is within reach of the whiskers. Perhaps mice can hear the sound of the motor moving and anticipate whisker touches. Mice can likely anticipate the reward by a small amount, which explains why whisking is elevated prior to reward consumption in data from the example mouse in Figure 2-3A. The solenoid valve that releases water makes a loud clicking sound. Reward consumption was defined as the first lick after the release of water and some mice showed elevated whisking prior to this lick, perhaps in response to the sound of the solenoid. When the solenoid opening time was used as a predictor in the regression analysis, the unique contribution to POm activity remained low, so we believe that POm truly does not represent rewards or reward anticipation, but does represent whisking movements during this time.

We show that approximately one fifth of POm neurons have a preference for whisker deflection. Direction selectivity has been seen across the whisker system, including in POm-projecting regions such as S1, zona incerta, and SpVi (Bruno and Simons 2002; Wilent and Contreras 2005; Furuta, Nakamura, and Deschenes 2006; Urbain and Deschênes 2007; Kaloti et al. 2016). Even if POm is not very touch responsive, it likely inherits direction selectivity from one of these regions. POm lacks local recurrent intranuclear circuitry and is likely unable to synthesize direction selectivity on its own (Ohno et al. 2012). We expected that the direction selectivity of POm-projecting regions might support strong touch responses to our directional,

multi-whisker stimuli, particularly since pole stimuli like ours strongly drive SpVi (Kaloti et al. 2016). POm, however, did not inherit the strong response, perhaps due to feedforward inhibition from zona incerta, an inhibitory subthalamic nucleus that receives input from SpVi (M. N. Williams, Zahm, and Jacquin 1994; Lavallée et al. 2005).

A similarly sized subset of POm neurons preferred fast or slow whisker deflections. We expected POm neurons to be sensitive to the velocity of whisker movement and were surprised that many of this velocity sensitive cells are actually suppressed by higher velocities. S1 cells, including cells in layer 5, which project to POm, have increasing firing rates with increased velocity (Bourassa, Pinault, and Deschênes 1995; Pinto, Brumberg, and Simons 2000; Wilent and Contreras 2004). Furthermore, S1 is theorized to be the main driver of POm neurons (Groh et al. 2014; Sherman and Guillery 2011). One reason POm would have neurons with an inverse relationship with velocity could be due to layer 5 cortical feedback via the zona incerta, a subthalamic nucleus that likely has strong inhibitory control over POm (Veinante, Lavallée, and Deschênes 2000; Barthó, Freund, and Acsády 2002; Trageser and Keller 2004). S1 activity can influence zona incerta firing rates (Barthó et al. 2007), which in turn could inhibit POm neurons. Thus, some velocity-sensitive neurons in layer 5 might directly excite POm neurons and induce a positive correlation with velocity in POm. Other velocity-sensitive neurons in layer 5 might project to zona incerta, which might project to other POm neurons, inducing a negative correlation with velocity in those neurons. Perhaps an inverse relationship with velocity should be expected of POm and the paralemniscal system. SpVi, a major trigeminal brainstem input to POm, has been found to have higher spike rates in response to slower velocity stimuli (Kaloti et al. 2016).

Approximately half of the POm neurons were negatively affected by one or more velocity of touch trial. A negative response to a touch trial might occur if cells are inhibited by the zona incerta from either feedforward mechanisms from SpVi or feedback from S1 (Lavallée et al. 2005; Veinante, Lavallée, and Deschênes 2000; Barthó et al. 2007).

Licking was the second largest contributor to POm activity. The contribution of licking in combination with the contribution of whisking to POm activity suggests that POm might be responsive to body movements in general. Body movement and facial movement signals have been shown to dominate the activity of much of the dorsal cortex and are even present in the rest of the brain (Musall et al. 2019; Stringer et al. 2019). It is possible that responsiveness to body movements allows sensory areas of the brain to integrate sensory information with motor output. Integrating these signals is essential for creating internal models of the external world and for moving and behaving appropriately in that world. Since POm is interconnected between somatosensory cortices and motor cortex, it might be a prime region for sensorimotor integration and may even broadcast these motor signals across sensory regions.

Alternatively, these movement signals might not represent movement per se, but rather internal states that often correlate with movement, such as arousal (Polack, Friedman, and Golshani 2013; Vinck et al. 2015). When mice are in states of high arousal, they also tend to make movements such as locomotion (Vinck et al. 2015). But locomotion and arousal signals in the brain are not the same, since high arousal states also occur in the absence of locomotion (Vinck et al. 2015). In our experiments, mice whisked the most on water reward trials. The ability of internal states such as arousal, behavioral engagement, and reward-modulated motivational states to alter activity in sensory brain regions allows animals to selectively engage with external stimuli when it is advantageous for them to do so. POm is a potential convergence

zone between external sensory signals from the brainstem and internal signals from cortical regions and might be involved in the integration of sensory stimuli with the animal's internal context.

## 2.4 Materials and Methods

### *2.4.1 Experimental Subjects*

We report data here from 9 adult mice of the C57BL/6J strain. These mice were obtained from Jackson Labs or bred (from parents of the Jackson Labs substrain) in the Columbia University animal facilities. Mice were group-housed and lived in a pathogen-free barrier facility. All experiments complied with the NIH Guide for the Care and Use of Laboratory Animals and were conducted under the supervision and approval of the Institutional Animal Care and Use Committee of Columbia University.

### *2.4.2 Surgeries*

Mice were stereotactically injected with a GCaMP-encoding virus (AAV5.CamKII.GCaMP6f, Penn Vector Core, Addgene) into POm between postnatal day 76 and 138. They were administered carprofen and buprenorphine. Bupivicaine was delivered at the craniotomy site. Mice were kept under isoflurane anesthesia during the procedure. A small craniotomy was made with a dental drill above POm (1.4 mm medial and 1.7 mm posterior to bregma) and 306 nL of a diluted virus was injected into POm with a pulled pipette (20-30 um diameter) fastened to a Nanoject III (Drummond). The virus had a titer of $10^{-13}$ and was diluted with artificial cerebrospinal fluid at a 1:2 dilution. The craniotomy was covered with artificial cerebrospinal fluid and sealed with cyanoacrylate glue (Vetbond) and the skin sutured. Mice were allowed to recover for 12 or more weeks to allow for viral expression.

An average of 19 weeks post injection, mice were implanted with a GRIN lens and a custom-designed stainless steel headplate (manufactured by Wilke Engenuity). They were administered carprofen and buprenorphine. Bupivicaine was delivered at the craniotomy site. Mice were kept under isoflurane anesthesia during the procedure. The scalp and fascia over the dorsal surface of the skull was removed. A craniotomy slightly larger than 1mm was made, centered at the site of viral injection. The tissue was aspirated out above POm and a 1mm GRIN lens (GRINTech NEM-100-25-00-860) lowered into position. The lens was attached to the skull with cyanoacrylate glue (Vetbond). Following lens implantation, we coated the skull with cyanoacrylate glue and then affixed the headplate to that with dental acrylic. Polypropylene snap-on caps (McMaster-Carr) were affixed to the surface of the enclosed headplate with additional dental acrylic to protect the lens surface. Mice were allowed to recover a minimum of 2 weeks.

### *2.4.3 Stimulus Apparatus*

The apparatus for delivering stimuli was built with a combination of optical posts (ThorLabs), custom laser cut acrylic pieces, custom 3D printed pieces, and an aluminum breadboard (ThorLabs). A custom milled stainless steel headfixing post sat above a custom 3D printed tube in which the mice stood for the experiment. A stepper motor (Pololu 1204) attached to a vibration dampening post (ThorLabs) rotated a horizontal custom laser cut acrylic arm with a vertical pole (0-80 allen wrench, Pololu) on each end (one through the whiskers, one as a counter weight). Water rewards (~10uL) were delivered through tubing from a reservoir to a stainless steel tube (McMaster-Carr) via the opening of a solenoid valve (The Lee Co. LFAA1209512H). Sounds, though not included in this analysis, were delivered through a speaker (Sparkfun) and produced by a custom LabView program.

The experimental paradigm was controlled by an Arduino Uno. It controlled the motor and solenoid valve, sent go signals to the speaker, and monitored licks via a capacitive touch sensor (Sparkfun) attached to the stainless steel tube. The Arduino Uno ran a custom program written in C and C++ in the Arduino IDE.

Analog signals from Arduino channels for the solenoid, capacitive touch sensor, whisker video synching LED (see Whisker Video and Analysis) were captured through a data acquisition board (National Instruments). The galvo motor signal from the scanning mirrors of the two-photon microscope was also collected into this data acquisition board (National Instruments) for data synchronization.

### *2.4.4 Experimental Design*

Mice were first habituated to human handling, head-fixation, and the two-photon microscope. They were handled daily for 5 days before they began habituation to the setup. Next, they were water restricted and each day prior to receiving water in the home cage, they sat head-fixed in the stimulus apparatus with the microscope objective in place above their head and the scanning mirrors of the two-photon microscope running for 30 minutes per day for three days. Experiments took place in total darkness within a custom-built light-tight box that encased the two-photon microscope. Each trial type was delivered randomly with random inter-trial intervals ranging from 2000ms to 4100ms.

There were 6 trial types: three different velocity pole deflections, one water reward, and two frequency bands of random noise sound trials. The sound trials did not contribute to POm activity, but were also not enough evidence to rule out sound-related responses, so were not included in this paper. Nevertheless, they were part of the experience of our experimental mice. On touch trials the pole was rotated through the whiskers from the rest position behind the

whisker field to a position past the forwardmost point the whisker field and then returned to the rest position out of reach of the whiskers. The radius of the pole rotation was placed 5-7mm from the face and the speed was calculated to create a fast (3100 degrees/second when 5mm or 2200 degrees/second 7mm from the face), medium (2000-1500 degrees/second), or slow (1100-800 degrees/second) deflection of a centrally located whisker when held at a position perpendicular to the surface of the whisker pad. Since mice had full control of their whiskers, the exact deflection velocity varied. On water reward trials, ~10ul of water was delivered through the lick port (stainless steel tube) and mice needed to lick to drink the droplet. Mice were not previously trained to drink water from lickports. Licking (via capacitive touch on the lickport) and whisking (via video recording) were recorded throughout.

### *2.4.5 Whisker Video and Analysis*

An infrared lamp was aimed at the mouse to illuminate the whiskers on both the contralateral and ipsilateral sides of the face relative to imaged POm. One camera (Sony PS3eye with M12 lens) was aimed diagonally at the contralateral whiskers from below. Another camera was aimed at the ipsilateral whiskers diagonally from below. A small LED was placed within view of both cameras and flashed at the start of each trials for the purposes of synching the video data with the Arduino signals. Videos with dropped frames were later discarded (these were always ipsilateral videos, likely due to the order of camera recording onset). Video was recorded at 125 frames/second with GUVCVIEW software.

For the contralateral side, whiskers were traced and angles recorded with the whisk package (Clack et al. 2012). We then took the median angle on each frame as the whisking angle. This signal was bandpass filtered at 4-25 Hz. A Hilbert transform was used to find the $\pi$ points and the 0 points of the whisker phase. Whisking amplitude was calculated by subtracting the 0

points from the π points. The times that the pole was within reach of or actively deflecting the whiskers were manually extracted from 6 trials for each mouse. Given slight variations in the angle of the headplate and the distance of the pole's radius to the face, these were individually recorded for each mouse. The whisking angles and amplitudes were discarded during these touch windows.

For the ipsilateral side, whisker motion was calculated. A region of interest over the whiskers was manually selected and then the average pixel intensity change within that region was calculated on each frame from the previous frame.

### *2.4.6 Two-Photon Imaging*

Two-photon imaging was conducted on a Sutter movable objective microscope under the control of the ScanImage software package (V. Iyer, Janelia Farms). Scanning during awake conditions was performed at 30 fps using a Chameleon Ultra II laser (Coherent) tuned to 940 nm, precompensated for group velocity dispersion and focused through a 16x/0.8NA water immersion lens (Nikon). Emitted light was collected with an HQ535/50 filter (Chroma) and GaAsP photomultiplier tubes (Hamamatsu Photonics). Thalamic neurons were imaged through a GRIN lens (GRINTech NEM-100-25-00-860) (778 x 778 μm field of view, 512 x 512 pixels).

The working distance, or distance from the bottom of the lens to the imaged neurons in the brain, of the GRIN lens was experimentally measured by immersing the bottom of the lens in a cuvette of fluorescein diluted in water. We aimed a microscope (Leica) perpendicular to the cuvette and took images with a microscope-attached camera (Q Imaging) while focusing the two-photon laser through the GRIN lens at different depths. We then measured the distance between the bottom of the lens and the point of fluorescence in the fluorescein when the two-photon objective was focused at the distance from the top of the lens that was used during *in vivo*

imaging. The working distance was determined to be approximately 50um from the bottom edge of the lens. We were able to measure working distance depths closer to the specifications from the GRIN lens manufacturer (250um) when we placed the two-photon objective at its working distance (3mm) from the lens surface (so that the microscope was focusing on the top surface of the GRIN lens). In practice, this arrangement did not yield images of neurons.

### *2.4.7 Histological Reconstruction*

Prior to the experiment, we took epifluorescence images of the surface of the GRIN lenses using an epifluorescence microscope (including a QImaging Rolera CCD camera and a low-magnification objective: Zeiss 10X/0.3NA) whose beam path intersected with that of our two-photon microscope, thus viewing the same view through the GRIN lens at a lower magnification as that used during two-photon imaging. After experiments, heads were placed in a 4% paraformaldehyde solution for 7 days in order to preserve the location of the lens within the tissue. After this time, the implant was removed and the brain was extracted. Brains were rinsed in phosphate-buffered saline for one day then sliced with a Vibratome sectioning system. Images were taken with a stereoscope (Nikon). We used the SHARP-Track analysis software (Shamash et al. 2018) to align the images with the Allen Brain Institute Mouse Atlas (Allen Institute for Brain Science 2015) and to mark the bottom edge of the empty space left by the GRIN lens. Next, we used custom MATLAB code to compute the location of the plane in atlas space located at the working distance from the lens. We determined where the points along this plane lay within the Allen Brain Institute Mouse Atlas and created a map of the thalamic nuclei within the field of view of our imaging. We overlaid this map onto epifluorescence images of the surface of the GRIN lenses and were able to match visible cues such as blood vessels and presence of GCaMP-labeled neurons across the epifluorescence images and two-photon videos. Thus, we

were able to identify which neurons were within POm and which were within other thalamic nuclei. We excluded neurons that may have been in POm but were too close to nucleus borders to be certain.

### *2.4.8 Data Analysis*

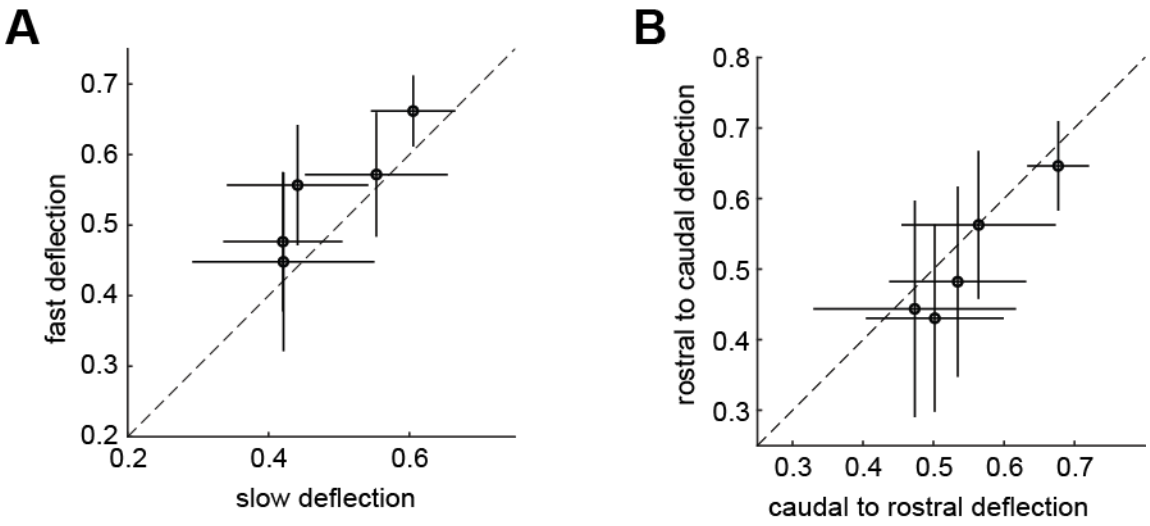
Two-photon videos were motion corrected and segmented with the Suite2P software package (Pachitariu et al. 2017). Segmented data was manually inspected and only the ROIs that were determined to be likely real neurons were kept. Frames in which 40% of identified neurons were reduced in calcium signal by 20% or greater within a single frame were excluded from analysis in order to eliminate possible contamination of motion artifacts in the calcium signal.

$\Delta F/F$ was calculated in two ways. In both, $\Delta F/F$ was calculated as $(F-F_0)/F_0$, where F is the fluorescence in the current time point and $F_0$ is the baseline signal. In the analyses in Figure 2-2A and B and to determine if POm neurons were responsive to touch, $F_0$ was defined as the average fluorescence of the cell in the 500ms period before stimulus onset, which emphasizes what of the fluorescence signal changed when the stimulus was applied. In the remaining analyses, including about touch preference, $F_0$ was calculated using the probability density estimate of a normal kernel function (using the MATLAB function ksdensity) over a 55 second sliding window.

For the regression analyses, whisking amplitude was threshholded to 30% of the max amount and converted into a binary whisking (1) or not whisking (0) signal so it could be better compared to the other predictors. All data was binned into 300ms bins to account for timing differences in the signals. The fluorescence was the average value across this time bin. For all regressions, we used the MATLAB function fitlm to try to predict the fluorescence values from a number of predictors. Each cell was modeled separately. For the bivariate regressions to predict

fluorescence from either whisking alone or touch alone, we only included data from touch trials and inter-trial intervals. For the multiple regression model, we included the entire experimental session. The whisking predictor was the average contralateral whisking amplitude. Whisking amplitude was interpolated across the time periods in which the pole was within reach of the whiskers, when whisking amplitude was not measurable. The touch velocity predictor was a categorical variable that was set to 1 when pole was within reach of the whiskers and moving at the fast velocity, 2 when the pole was at the medium velocity, and 3 when the pole was at the fast velocity. The reward predictor was defined as 1 at the time of the first detected lick after the solenoid released the water reward. The lick predictor was defined as the number of licks within the 300ms time bin. These predictors were normalized between 0 and 1 prior to being used to predict calcium. The $R^2$ value adjusted for the number of predictors was reported. To calculate the unique contribution of each predictor, that predictor was replaced in the model by a version that was shuffled in time. This was done 100 times for each predictor and the average $R^2$ was taken from the resulting 100 $R^2$ values. The unique contribution was the difference between the full model $R^2$ and this averaged $R^2$.

## 2.5 Supplemental Material




**Supplemental Figure 2-5 Ipsilateral whisking motion during touch trials**
A) Average ipsilateral normalized whisking motion for fast and slow pole deflections. Error bars indicate SEM. n = 5 mice. B) Same as B but for caudal-to-rostral and rostral-to-caudal deflections.

# Chapter 3: Posterior medial thalamus does not learn whisker-based stimulus-reward associations

## 3.1 Introduction

The ability to learn from past experience requires the brain to flexibly change neural activity according to sensory evidence. In the cortex, neural representations of stimuli in sensory discrimination tasks are enhanced by learning (Beitel et al. 2003; Fritz et al. 2003; David, Fritz, and Shamma 2012; Goltstein et al. 2013; Poort et al. 2015; Henschke et al. 2020). Feedback signals from higher-order cortex can modify learning in primary sensory cortex (Banerjee et al. 2020; Liu et al. 2020). The apical dendrites of layer 5 cells that fall within cortical layer 1 are thought to receive feedback signals and might be able to use these feedback signals to enhance incoming sensory signals (Felleman and Van Essen 1991; M. E. Larkum, Senn, and Lüscher 2004; M. Larkum 2013). Our lab recently demonstrated that layer 5 apical dendrites in primary somatosensory cortex (S1) become more selective to both rewarded and unrewarded air puffs to the whiskers after learning the sensory-reward associations (Benezra et al., submitted).

S1 apical dendrites of layer 5 and layer 2/3 cells receive input from secondary somatosensory thalamus (the posterior medial nucleus, POm) (Petreanu et al. 2009). We previously demonstrated that POm is a stronger input to layer 2/3 apical dendrites than some cortical regions (Zhang and Bruno 2019). POm is also capable of increasing the gain of sensory responses within layer 5 cells (Mease, Metz, and Groh 2016). Furthermore, POm can facilitate plasticity-related NMDAR-dependent plateau potentials in layer 2/3 apical dendrites (Gambino et al. 2014), suggesting a role for POm in cortical learning. Indeed, there are effects of learning within POm's connection to S1. After mice learn whisker-based sensory-reward associations,

POm synapses onto layer 5 cells were potentiated (Audette et al. 2019). These studies suggest that POm may be the source of increased selectivity to task relevant sensory stimuli seen in S1. How POm activity changes over the course of learning remains an open question.

Studies of the POm projection to layer 1 of S1 have shown that POm is more active when mice are licking and responding to learned rewarded stimuli (El-Boustani et al. 2020; LaTerra et al. 2020). In Chapter 2, we showed that POm activity in naïve mice is best explained by movements such as whisking and licking, even more than touch. To determine whether POm responses might change over learning and explain selectivity changes within S1, we trained mice on a Pavlovian conditioning task in which one direction of air puff signaled reward while the other air puff was unrewarded. We demonstrate that POm activity becomes elevated after the rewarded stimulus, but that these changes in activity are explained by whisking and licking movements. The contribution of reward-associated sensory stimuli to POm activity does not change with learning. We propose a model in which following a strategy of encoding movements, particularly those associated with exploration and motivation such as whisking and licking, POm may be able to facilitate the learning of relevant sensory features within the cortex.

## 3.2 Results

### *3.2.1 A Pavlovian conditioning task for associating air puff direction with reward*

To assess POm responses to learned stimulus-reward associations, we used a behavioral task previously developed in the lab (Benezra et al., submitted) that presents head-fixed mice with air puffs in two directions: one vertically aimed air puff from below the whisker field and one horizontally aimed air puff from in front (Figure 3-1A). For each mouse, one of these air puffs (the conditioned stimulus, CS+) was followed by a water reward 500ms after the offset of the puff. The other air puff (unconditioned stimulus, CS-) was not followed by reward. Four out

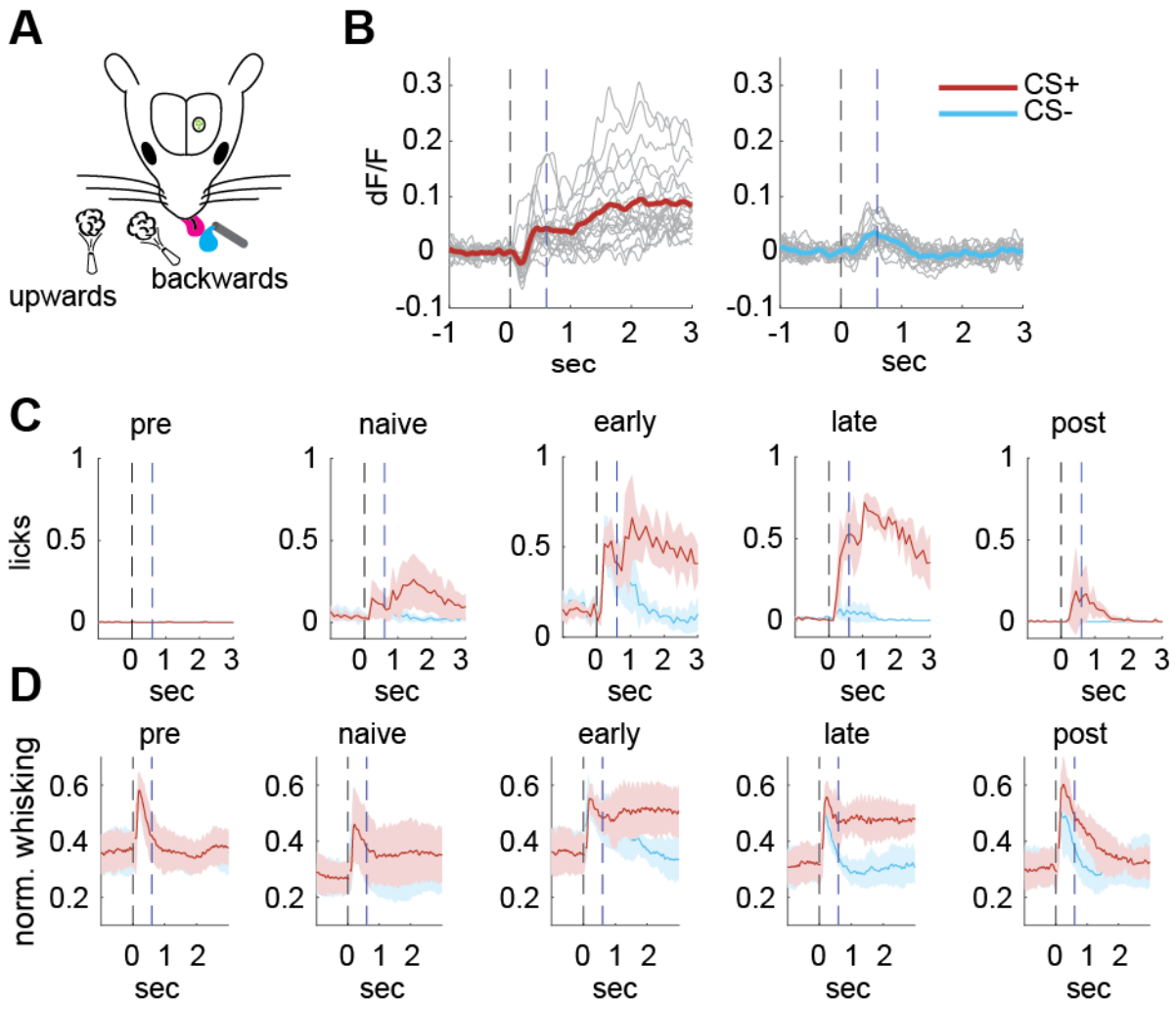



**Figure 3-1 Learning touch-reward associations increases whisking, licking, and POm selectivity on CS+ trials**

A) Experimental setup. Calcium imaging of GCaMP6F in POm neurons through a GRIN lens in awake mice. Air puffs are delivered upwards from below the whiskers or backwards from in front of the whiskers. One direction of air puff is followed by water reward (CS+), one is not (CS-). B) Event-triggered averages of POm calcium activity for an example mouse (n = 21 cells). Left, CS+ trials; right, CS- trials. Gray, individual neurons; red and blue, means. Dotted black lines indicate air puff onset. Dotted blue lines indicate reward onset (CS+) or omission (CS-). C) Average licks across learning (n = 3 mice). Blue, CS+ trials; red, CS- trials. Shading indicates standard error. D) Same as C for normalized contralateral whisking motion.

of 9 mice learned the task within a month of daily training (Supplemental Figure 3-4, Supplemental Figure 3-5). One of these was only given 2 weeks due to extenuating circumstances but successfully learned (Supplemental Figure 3-4). Another had a mistargeted

GRIN lens above the lateral posterior nucleus instead of above POm (data not shown) and was excluded from the dataset. Mice were considered to have learned the task if the 95% confidence interval for their overall performance exceeded 50% correct (anticipatory licks present on CS+ trials and no anticipatory licks present on CS- trials were counted as correct, otherwise the trial was incorrect) for three consecutive days and if on each of these days the percent of CS- trials with anticipatory licks was below 40%. The timescales of learning for our mice contrasts with the short learning timescales seen previously in our lab (7-9 days, Benezra et. al., submitted). It is possible that the implantation of the GRIN lens or the age of the mice (mean age of our mice was postnatal day 285, mean age in Benezra et al. was 95) made learning more difficult.

Training began with a pre-learning session, which did not administer rewards after the CS+. On the naïve day, the first day following the pre-learning session, water rewards were introduced into the paradigm. As mice learned the association, they naturally began to lick during the delay period in anticipation of reward (Figure 3-1C). We defined the early learning stage as when mice exhibited anticipatory licking on 70% or more of both CS+ and CS- trials. By the late learning stage, mice showed anticipatory licking on more than 80% of CS+ trials and fewer than 40% of CS- trials. After mice reached the criterion for learning described in the previous paragraph, they performed a post-learning session during which rewards were omitted, similar to the pre-learning session. Mice often reduced their licking on this session (number of licks on the late-learning session ranged from 1705 to 2327, number of licks on the post-learning session ranged from 6 to 325). Over the course of learning, mice began to whisk in a trial-related way. Whisking following the CS+ and CS- was similar on the pre-learning stage (Wilcoxon rank sum test, $p > 0.05$ for 2 of 3 mice). Mice whisked more on CS+ trials than on CS- trials by the late learning stage (Wilcoxon rank sum test, $p < 10^{-18}$ for all 3 mice) (Figure 3-1D).

Following the post-learning session, mice performed one or more retraining sessions to ensure that they still knew the reward contingencies. After successful performance on a retraining session, two mice had their whiskers trimmed. The following day, mice were tested with the behavior to assess whether performance depended on the whiskers. Performance was reduced to chance (95% confidence interval included 50%) or worse than chance, suggesting that the behavior is dependent on whisker sensation of the air puffs (Supplemental Figure 3-4).

### *3.2.2 POm responses change with learning, but are not selective to whisker stimuli*

To assess POm responses over the course of learning, we used viral vectors to express GCaMP6F in POm and used two-photon microscopy to image neurons in the same region daily through GRIN lenses while mice were trained on the behavior (n = 78, 70, 76, 86, 89 POm neurons on pre-, naïve, early, late, and post-learning sessions, respectively, across 3 mice). These mice were from the group included in Chapter 2, so we knew that prior to exposure to this experimental paradigm, POm activity was better explained by whisking than by touch stimuli (see Chapter 2, section 2.2.3). POm activity immediately following the CS+ increased after learning (naïve vs. late, $p = 0.01$, Wilcoxon rank sum test; Figure 3-1B, Supplemental Figure 3-6). These responses appeared similar to those seen in Chapter 2 for the reward trial. This effect did not occur on the CS- trials (naïve vs. late, $p = 0.17$, Wilcoxon rank sum test). On the late learning session, POm activity was higher on CS+ trials than on CS- trials ($p<10^{-3}$, Wilcoxon rank sum test; Supplemental Figure 3-6), but this effect disappeared in the post-learning session ($p = 0.10$, Wilcoxon rank sum test).

The most dramatic changes in POm activity appear to be after the reward delivery. To determine whether learning changed POm responses to the air puff stimuli, we calculated a selectivity index (SI) across the 500 ms window from air puff onset (Figure 3-2, see 3.4.6 Data Analysis). In the pre-learning stage, 3.8% of the neurons were selective for either the CS+ or CS- (3 of 78 neurons; Wilcoxon rank sum test, CS+ response vs. CS- response, deemed significant if $p<0.05$) and the distribution centered around zero (median SI = 0.004). After exposure to rewards, in the naïve stage of learning, selectivity was similarly low (10%, 7 of 70 neurons, Wilcoxon rank sum test, CS+ response vs. CS- response, deemed significant if $p<0.05$; median SI = -0.07). By late learning, selectivity remained centered around zero (median SI = -0.02) and 25.6% of the neurons were selective for the CS+ or CS- (22 of 86 neurons, Wilcoxon rank sum test, CS+ response vs. CS- response, deemed significant if $p<0.05$). This increase was not significant (|SI| naïve vs. |SI| late: $p = 0.26$, Wilcoxon rank sum test). These results demonstrate that POm neurons do not develop selectivity for the CS+ or the CS- over the course of learning.

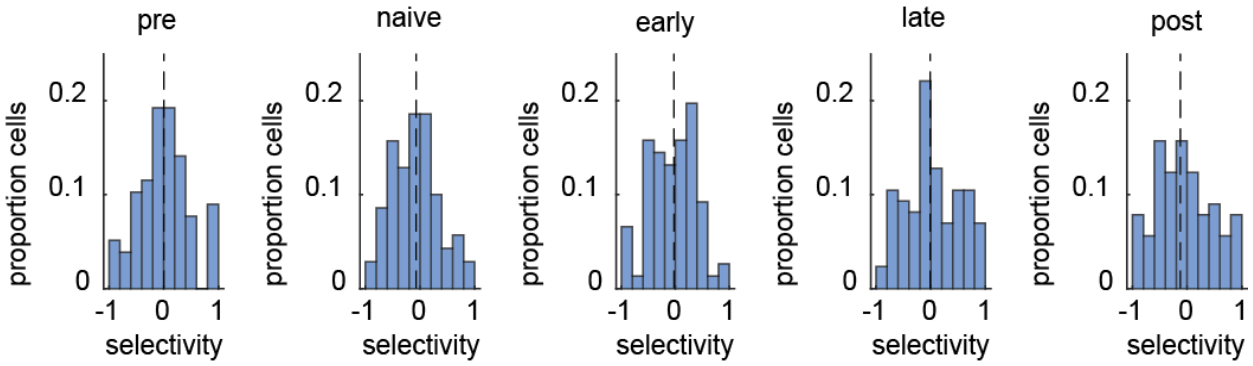



**Figure 3-2 POm selectivity does not change over learning**
Selectivity index of POm neurons across learning. -1 indicates preference for the CS-, 1 indicates preference for the CS+. Dotted lines, median value.

### *3.2.3 Movement dominates POm activity throughout all stages of learning*

POm did not increase in selectivity to the air puff stimuli with learning, but did increase in activity on CS+ trials. These trials also showed increases in whisking and licking. As seen in Chapter 2, movement signals, particularly whisking, best explained POm activity in mice that were not engaged in a behavioral task. To determine whether movement signals similarly dominated POm during learning, we again fit a multiple linear regression model to each neuron attempting to explain its activity with whisking, the CS+, the CS-, licking, and rewards. (On sessions with no rewards or no licking, we omitted these predictors from the model.) The amount of variance in POm fluorescence that was explained by these models ranged from 0 to 24% (Figure 3-3A), which is typical of these types of single trial models (Ramirez et al. 2014; Musall et al. 2019; Peron et al. 2015).

To determine the contribution of each predictor to POm activity, we performed a shuffling procedure as described in Chapter 2. This analysis confirmed that whisking and licking best explained POm activity across learning (Figure 3-3B). Neither the air puff stimuli nor rewards contributed much to POm activity. Though the contributions were small, there was a significant increase in the contribution of the CS+ (naïve median = $-9.2 \times 10^{-5}$, late median = $4.8 \times 10^{-5}$; Wilcoxon rank sum test, $p < 10^{-3}$) and in the contribution of rewards from naïve to late

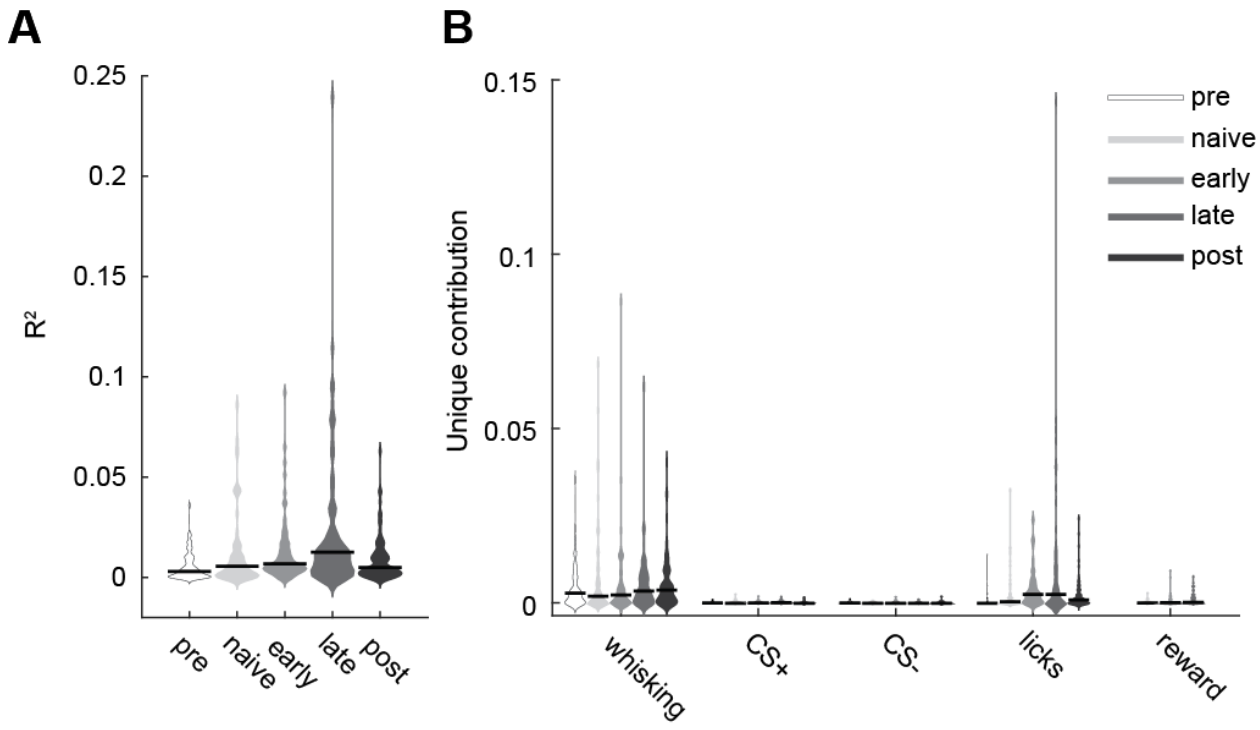



**Figure 3-3 Modulation of POm by movement is stable across learning**
A) Proportion of variance explained (adjusted $R^2$) by multiple linear regression for each cell across learning (n = 70-89 neurons from 3 mice). Black lines show median $R^2$ value of the population. B) Unique contributions of each regressor in the model for each cell across learning. Calculated from the $R^2$ of the cell's model with that regressor shuffled subtracted from the $R^2$ for that cell. Black, medians.

learning (naïve median = $-3.1 \times 10^{-5}$, late median = 0.0002; Wilcoxon rank sum test, $p < 10^{-5}$). This increase was not present for the CS- (naïve median = $-5.8 \times 10^{-5}$, late median = $-5.4 \times 10^{-5}$; Wilcoxon rank sum test, p = 0.67). These results suggest that even though movement dominates POm across learning, there may also be a very subtle increase in the amount of information about rewarded sensory stimuli and about rewards that POm acquires when mice have learned the task. However, the CS+ contributions to POm did not persist in the post-learning session. The CS+ contribution to POm activity in the pre- and post-learning sessions (pre-learning median: $-4.5 \times 10^{-5}$, post-learning median: $-7.1 \times 10^{-5}$, Wilcoxon rank sum test, p = 0.56) This result

demonstrates that the increase in contribution of the CS+ is either not robust or it is transient and lost when rewards are removed in the post-learning session.

The contribution of licking to POm activity increased over learning. In the late learning stage, licking contributed more to POm activity than during the naïve learning stage (naïve median contribution = 0.0004, late median contribution = 0.0024; Wilcoxon rank sum test, $p < 10^{-3}$). A contribution of licking may be difficult if not impossible to detect in the pre- and post-conditioning sessions due to the lack of licks in those session. Nevertheless, the naïve stage exhibits a substantial number of licks albeit fewer than the early and late stages, suggesting we should have been able to detect a lick contribution if one existed during the naïve stage (Fig.5D). (Average licks detected on pre-, naïve, early, late, and post-learning sessions was 11, 1,061, 4,288, 2,001, and 145, respectively, and was never zero for any of the mice). The contribution of licking did not change from the early learning stage to the late learning stage (early median contribution = 0.0024; Wilcoxon rank sum test, $p = 0.54$), which suggests that increased contribution of licking might not be due to learning the behavior, but rather due to a larger number of licks on those sessions.

On both the early and late learning stages, whisking and licking contributions were not significantly different. As expected from the results in Chapter 2, whisking contributed more to POm activity than licking on the naive learning stage (median whisking contribution = 0.0019, median licking contribution = 0.0004, Wilcoxon sign rank test, $p < 10^{-3}$). Whisking and licking contributions were not significantly different on the early and late learning stages (early learning: median whisking contribution = 0.0022, median licking contribution = 0.0024, Wilcoxon sign rank test, $p = 0.53$; late learning: median whisking contribution = 0.0034, median licking contribution = 0.0024, Wilcoxon sign rank test, $p = 0.58$). The contribution of whisking to POm

activity remained stable across learning and was not different between the naïve and late learning sessions (naïve median contribution = 0.0012, late median contribution = 0.0034; Wilcoxon rank sum test, $p = 0.44$). These results suggest that both movements, not just whisking, best explain POm activity. Over the course of learning stimulus-reward associations, POm representations are stably explained by these movements. Since these movements are elevated on trials with rewarded stimuli, they may indicate internal states of arousal, task engagement, or motivation.

## 3.3 Discussion

Here we show that POm representations are stable across learning. Movements such as whisking and licking dominate neural activity in POm, even after mice have learned a sensory-reward association. Furthermore, POm neurons do not increase in their selectivity for the air puff stimuli. These results surprised us, since previous work in which mice learned to associate a single air puff with rewards demonstrated that POm synapses onto layer 5 cells of S1 were potentiated (Audette et al. 2019). We expected POm representations to change while mice learned the associations. In the same behavioral task used here, our lab has shown that apical dendrites of layer 5 neurons increase in selectivity for both the CS+ and the CS- over the course of learning (Benezra et al., submitted). Some of these apical dendrites receive input from POm (Petreanu et al. 2009), but our results reveal that POm is not the source of this increased selectivity in S1.

Elevated activity in S1-projecting POm axons has been previously reported during licking in learned behavioral tasks (El-Boustani et al. 2020; LaTerra et al. 2020). We confirm that POm somata, which often do not always project to S1 (Ohno et al. 2012), exhibit lick-related activity. Interestingly, S1-projecting POm axons were also elevated during the response period of a behavioral task even when mice were trained to suppress licking as their "response" (LaTerra

et al. 2020). Here, POm activity could be modulated by either goal-directed behaviors or other body movements such as whisking that may not have been monitored. Our data show that POm representations of whisking and licking remain stable over learning, which suggests that POm activity may not be representative of a learned goal-directed response. Whether POm is modulated by body movements such as whisking and licking or by internal states that promote these body movements, which might also be involved in a suppression of licking response, remains an open question.

Perhaps by following state-related movements like whisking and licking, POm might be able to support plasticity within the cortex. Activating POm has been shown to induce NMDAR-mediated plateau potentials in layer 2/3 apical dendrites of S1 cells (Gambino et al. 2014). Furthermore, silencing POm prevented the development of a long-term potentiation effect seen in layer 2/3 cells after a rhythmic whisking stimulation protocol (Gambino et al. 2014). These data suggest that POm activity could play a role in the facilitation of plasticity within the cortex. If POm is activated by movements such as whisking and licking, which we demonstrate happen more on rewarded trials, it could facilitate plasticity within the cortex. The elevated POm activity during whisking and licking could disinhibit layer 2/3 apical dendrites in S1 and allow for plasticity of other inputs to occur. Disinhibition of layer 2/3 apical dendrites by POm activity has been seen *ex vivo* (L. E. Williams and Holtmaat 2019). This effect could be strengthened by the potentiation of synapses from POm to layer 5, which subsequently potentiates layer 2/3, as seen *ex vivo* after sensory-reward association training (Audette et al. 2019). Thus, POm activity during movements associated with exploration and motivation could allow the cortex to learn about sensory features in a goal-directed way.

## 3.4 Materials and Methods

### *3.4.1 Experimental Subjects*

The three mice described in this dataset as well as the other 6 mice trained on the behavior first underwent the procedures and experiments described in Chapter 2.

### *3.4.2 Behavioral Apparatus*

The behavioral apparatus was built by Sam Benezra and used for the same behavioral training as described previously (Benezra et al., submitted). Items on the apparatus were assembled on optical posts (ThorLabs) onto an aluminum breadboard (ThorLabs). Tubing connected a compressed air line to two solenoids (SMC). From the solenoids, tubing connected to wide pipette tips, one aimed from in front of the mouse towards the back and one aimed from below the mouse towards the top. The air puff pipettes were aligned such that their air paths crossed perpendicularly at whisker C2. They were aimed slightly away from the mouse to avoid air hitting the face. A masking sound was created with a third pipette underneath the mouse and aimed away, connected to a compressed air line that was open for the duration of the experimental sessions. A lickport made of a stainless steel tube was connected to tubing that led to a solenoid (The Lee Co. LFAA1209512H). When this solenoid was opened, water from a reservoir released ~10ml of water. An Arduino Uno controlled this solenoid with custom code written in C and C++ in the Arduino IDE.

### *3.4.3 Behavioral Task*

After water restriction and participation in the experiments in Chapter 2. Mice began behavioral training under the two-photon microscope. The behavioral task exactly matched what was reported previously, except that our mice spent longer training than 9 days and we did not

have a repeated exposure group (Benezra et al., submitted). These methods are copied below, lightly edited to match the procedures used here:

> Animals were given ~1 mL of water per day for the duration of training. Mice were head restrained in a custom-made behavioral apparatus by positioning the body in a 3D-printed chamber and fastening the head plate to metal posts flanking the chamber. Air puff stimuli (10 psi, 100 ms) were delivered from two nozzles positioned toward the distal tips of the whiskers, in either the rostrocaudal or ventrodorsal direction. Nozzles were oriented to prevent air jets from stimulating other parts of the face. One of these directions (CS+) was paired with a water reward (10 μL), delivered through a lick port 0.5 seconds after the stimulus. Approximately 180 stimuli were presented over the course of a 30-minute imaging session (8-12-s intertrial interval). The probability of CS+ or CS- delivery was 50%. In preliminary experiments, Benezra et al. found that an auditory mask helped prevent mice from exploiting auditory cues to discriminate the two stimuli: a third air nozzle was positioned close to the mouse and was active throughout the session.
>
> During the first session (pre-learning), stimuli were delivered in the absence of reward to assess neural and behavioral responses in naive animals. In the following days, the CS+ was paired with reward. Licks for rewards were detected with a capacitance-based touch sensor (Sparkfun). A trial response was registered when one or more licks were elicited within a 0.5-second response window following the stimulus and before reward delivery. To determine whether behavioral performance was above chance, we computed 95% confidence intervals using the 'binofit' function in MATLAB. During the final session (post-learning), stimuli were delivered in the absence of reward. Behavioral experiments were performed with the Arduino-based OpenMaze open source behavioral system, whose designs are fully described at www.openmaze.org. Whisking was monitored at 125 fps with a camera (Sony PS3eye) and automatically tracked using published software (Clack et al., 2012).

Mice were considered to have learned the task if the 95% confidence interval for their overall performance exceeded 50% correct (anticipatory licks present on CS+ trials and no anticipatory licks present on CS- trials were counted as correct, otherwise the trial was incorrect) for three consecutive days and if on each of these days the percent of CS- trials with anticipatory licks was below 40%. After three days of conditioning with these criteria met, mice proceeded to the post-learning session. Following the post-learning session, mice performed one or more retraining sessions to ensure that they still knew the reward contingencies. After successful performance on a retraining session, two of the mice had their whiskers trimmed. The following

day, mice were tested with the behavior to assess whether performance depended on the whiskers.

### *3.4.4 Whisker Video and Analysis*

Similar to section 2.4.5 Whisker Video and Analysis. Here we used solely contralateral whisker motion. In all analyses, whisker motion during the 100ms of the air puff stimuli was excluded. During this time window, the whiskers were moved by the air puff, so whisking could not be reliably measured by changes in motion.

### *3.4.5 Two-Photon Imaging*

As described in section 2.4.6 Two-Photon Imaging. Daily imaging was performed as mice learned the behavior.

### *3.4.6 Data Analysis*

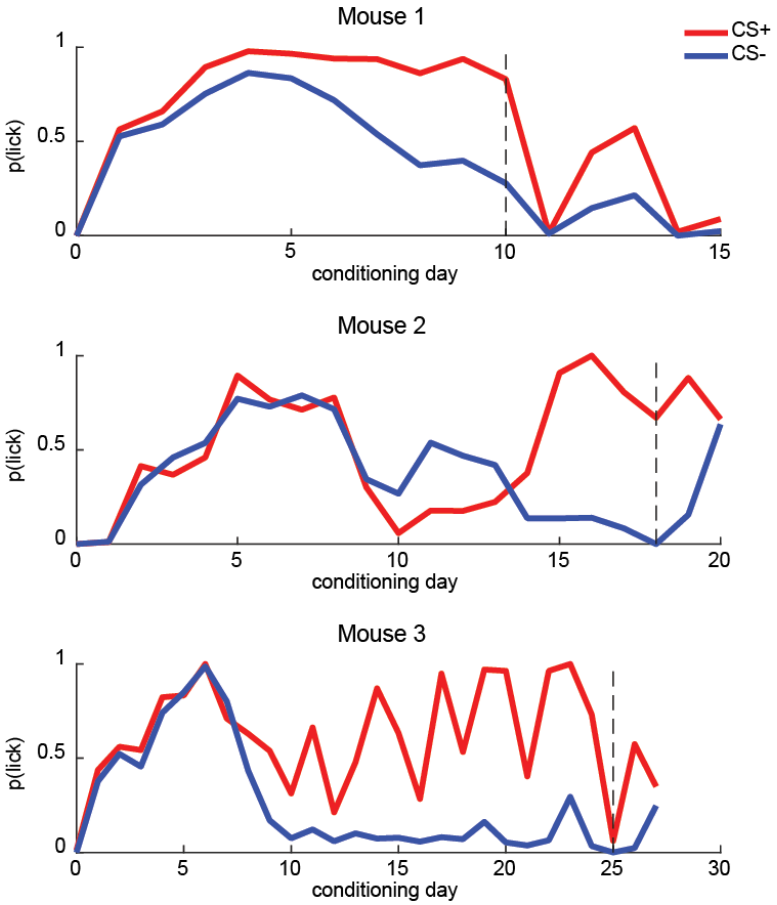
Similar to section 2.4.8 Data Analysis. ΔF/F was calculated in two ways. In both, ΔF/F was calculated as $(F-F_0)/F_0$, where F is the fluorescence in the current time point and $F_0$ is the baseline signal. In most of the analyses, $F_0$ was defined as the average fluorescence of the cell in the 500ms period before stimulus onset, which emphasizes what of the fluorescence signal changed when the stimulus was applied. In the regression analyses, $F_0$ was calculated using the probability density estimate of a normal kernel function (using the MATLAB function ksdensity) over a 55 second sliding window.

For the selectivity index (SI) for each neuron, the mean stimulus-aligned ΔF/F was computed across the CS+ or the CS- trials. SI was defined as $(F_{CS+} - F_{CS-}) / (F_{CS+} + F_{CS-})$, in which $F_{CS+}$ and $F_{CS-}$ are the mean stimulus-aligned amplitudes (ΔF/F) to the CS+ and CS- within

the first 500 ms, respectively. This yielded values that range from -1 (exclusively CS- responsive) to 1 (exclusively CS+ responsive).

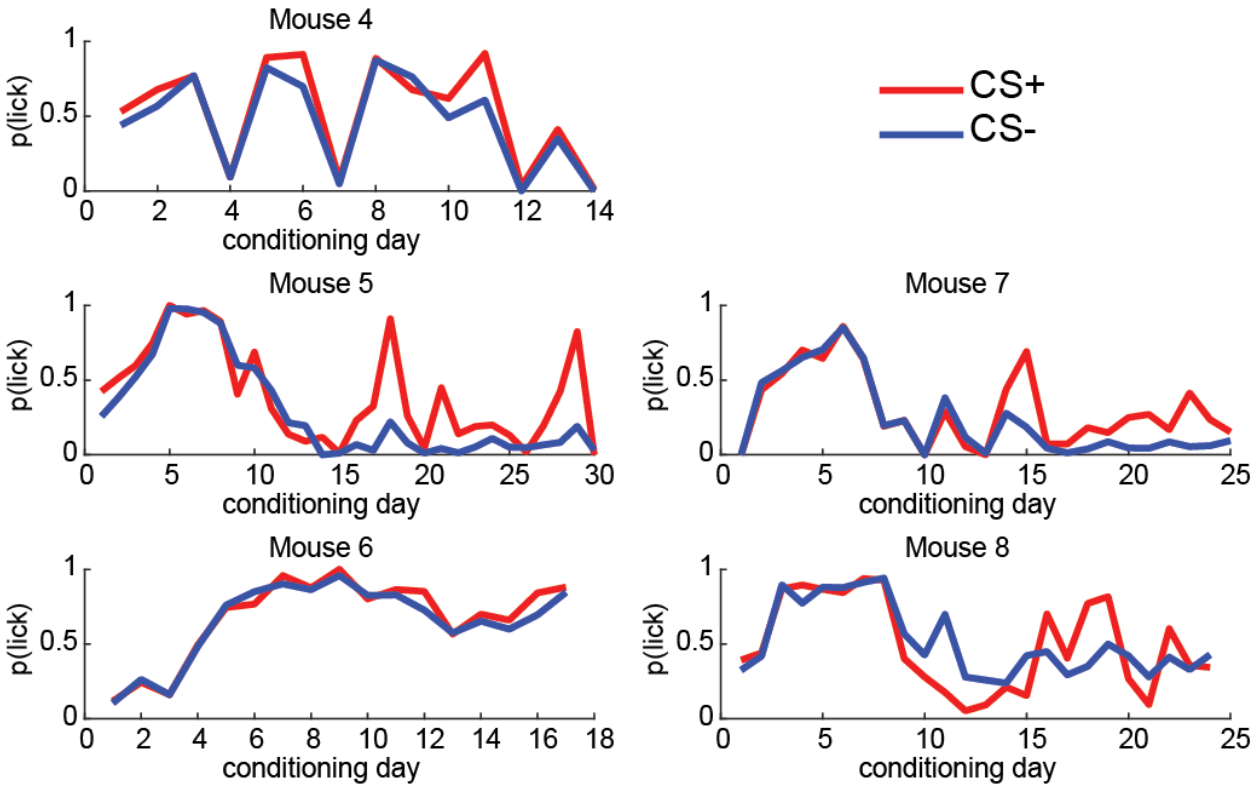
For the regression analyses, whisking motion was threshholded to 45% of the max amount and converted into a binary whisking (1) or not whisking (0) signal. All data was binned into 500ms bins to account for timing differences in the signals. The fluorescence was the average value across this time bin. For all regressions, we used the MATLAB function fitlm to try to predict the fluorescence values from a number of predictors. Each cell was modeled separately. In the multiple regression models, we included the entire experimental session. The whisking predictor was the average contralateral whisking motion. Whisking motion was interpolated across the 100ms when the air puff was delivered. The CS+ and CS- predictors were categorical variables that were set to 1 during the 100ms time window that the air solenoids were open. The reward predictor was defined as 1 at the time of the first detected lick after the solenoid released the water reward. The lick predictor was defined as the number of licks within the 300ms time bin. These predictors were normalized between 0 and 1 prior to being used to predict calcium. The $R^2$ value adjusted for the number of predictors was reported. To calculate the unique contribution of each predictor, that predictor was replaced in the model by a version that was shuffled in time. This was done 100 times for each predictor and the average $R^2$ was taken from the resulting 100 $R^2$ values. The unique contribution was the difference between the full model $R^2$ and this averaged $R^2$.

## 3.5 Supplemental Material

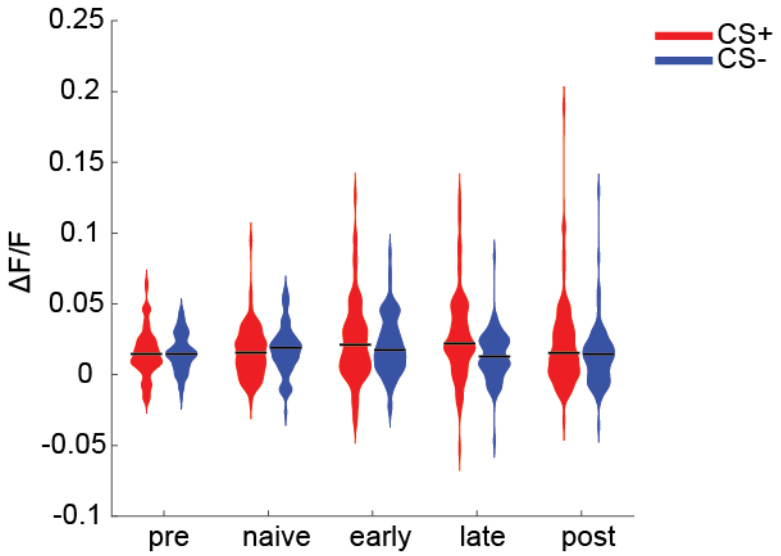



**Supplemental Figure 3-4 Mice can learn the conditioning task within one month**

Learning curves from the mice who learned the air puff conditioning task. Performance shown in percentage of CS+ (red) or CS- (blue) trials with anticipatory licks. Day 0 is the pre-conditioning day. Dotted lines indicate post-conditioning day. After the post-conditioning day, mice were again presented with the full task with rewards. After the penultimate conditioning day, the whiskers of Mouse 2 and Mouse 3 were trimmed. Performance on the final day (day 20 and 27, respectively) demonstrates that these mice used their whiskers to discriminate between the CS+ and CS-.




**Supplemental Figure 3-5 Not all mice learn the Pavlovian conditioning task**
Learning curves from the mice who did not learn the air puff conditioning task. Performance shown in percentage of CS+ (red) or CS- (blue) trials with anticipatory licks. Days shown are the first day of conditioning (after the pre-learning session) through the last day of conditioning (prior to the post-learning session).




**Supplemental Figure 3-6 POm activity becomes elevated on CS+ trials**
Average ΔF/F for each cell on CS+ trials and CS- trials (1500ms window from air puff onset)

# Chapter 4: Conclusion

## 4.1 Summary

The role of secondary somatosensory thalamus (POm) has long been mysterious. Its position in ascending pathways of whisker information suggests that it should relay touch information to the cortex; yet in most manners, VPM does a better job at relaying whisker signals to the cortex than POm (Diamond, Armstrong-James, and Ebner 1992; Moore et al. 2015; Urbain et al. 2015). Additionally, POm is interconnected with motor, higher-order somatosensory, and association cortices (Aldes 1988; Bucci, Conley, and Gallagher 1999; Rubio-Garrido et al. 2009; Liao et al. 2010; Hooks et al. 2013; Olsen and Witter 2016). These cortical connections provide a substrate that may allow POm to respond to or integrate contextual signals such as movements or rewards with touch.

We show that POm activity does exhibit weak touch preferences in awake mice. Yet, most of the activity in POm is best explained by movements such as whisking and licking. Rewards do not contribute to POm activity and POm does not develop selectivity for reward-associated whisker stimuli. Instead, both whisking and licking modulate POm activity before, throughout, and after learning. Since these movements are often carried out in states of exploration or motivation, we propose a model in which the representation of movements within POm may facilitate learning sensory features in cortex by creating a window for plasticity around relevant stimuli.

## 4.2 Current status of understanding POm

### *4.2.1 Representations of touch in POm*

VPM has more spatially and temporally precise responses to touch and tracks both fast and slow aspects of whisking rather than just slow aspects (Diamond, Armstrong-James, and Ebner 1992; Moore et al. 2015; Antonini et al. 2020). But there are other aspects of whisker touch that VPM does not excel at, such as direction tuning. VPM has angular sensitivity, but S1 is better tuned than VPM (Bruno and Simons 2002). Direction tuning arises when neurons respond well to similar angular deflections of each of the whiskers in their multi-whisker receptive fields, reflecting a preference for the direction of the object that moves through the whiskers. Whisker-related areas that project to POm, such as SpVi, S1, secondary somatosensory cortex, and superior colliculus have more pronounced direction tuning than VPM (Roger and Cadusseau 1984; Bourassa, Pinault, and Deschênes 1995; Liao and Yen 2008; Hemelt et al. 2010). These data suggest that POm might also have stronger direction tuning than VPM, but whether direction tuning is present in POm was not known prior to the work we presented here. We demonstrate that 21% of POm neurons have direction selectivity. In addition to direction tuning, S1 also exhibits stronger velocity tuning than VPM. VPM neurons increase their initial firing rates to increasing velocities of whisker stimuli, but do not change their overall firing rate (Pinto, Brumberg, and Simons 2000). S1, however, does increase firing rates to increasing velocity (Pinto, Brumberg, and Simons 2000). While previously unknown, we demonstrate that 22% of POm neurons have velocity preferences. The POm population as a whole does not prefer faster velocities, however, which is unexpected given S1 preferences (Pinto, Brumberg, and Simons 2000; Wilent and Contreras 2004). Interestingly, SpVi neurons prefer slower velocities while S1 neurons prefer faster velocities (Kaloti et al. 2016; Pinto, Brumberg, and Simons 2000).

The fact that the POm population is mixed suggests a possible way to determine which POm neurons are more influenced by ascending brainstem inputs and which POm neurons are more influenced by top-down cortical input. These touch preferences within POm suggest an interesting role for POm in touch processing. While VPM and the lemniscal pathway may be a source of whisker touch information, including reafference from whisking movements, POm and the paralemniscal pathway may represent more external cues about touch. POm may preferentially encode aspects of the encountered object, such as its direction and velocity of movement, rather than touch parameters related to the mouse itself such as details of the individual whiskers that are touched or the reafferent whisking signal.

### *4.2.2 Representations of movement in POm*

POm and the paralemniscal system had previously been theorized to form a movement-specific pathway in which movement information about the whiskers was sent to the cortex (Yu et al. 2006). Integrating movement signals could allow the brain to calculate where the whiskers were in space when incoming touch signals arrived from the lemniscal system in order to locate objects. However, only VPM, not POm, is modulated by whisker phase, which would be needed to make this calculation (Moore et al. 2015). POm has been shown to be modulated by slower components of whisking such as whisking amplitude (Petty et al. 2020). We expand upon this knowledge by directly comparing touch and whisking responses in POm. The contribution of whisking activity to POm dwarfed that of touch.

It remains under debate whether the whisking signal is movement-related or indicative of a movement-correlated internal state such arousal (Polack, Friedman, and Golshani 2013; Vinck et al. 2015). We show that not only whisking, but also licking, particularly when there is a lot of licking data as in Chapter 3, contribute to POm activity. Whisking and licking movements are

both movements that correlate with internal state and motivation (Watanabe et al. 2001; Vinck et al. 2015). Furthermore, POm activity is correlated with pupil, a behavioral measure of arousal (Aston-Jones and Cohen 2005; Eldar, Cohen, and Niv 2013; Reimer et al. 2016; Petty et al. 2020). Interestingly, when comparing firing rates during an active, non-whisking brain state and a whisking state, POm did not have a significant increase, but VPM did (Urbain et al. 2015). These data all indicate evidence that POm signals may be more about internal states than about movements. However, we attempted to use rewards and rewarded stimuli to modulate internal states and the results were not clearly in favor of the internal state hypothesis. Rewarded stimuli have been known to cause changes in pupil dilation and arousal (Rudebeck et al. 2014). While we saw whisking on water reward trials, we did not see much contribution, if any, of rewards to POm activity. Furthermore, in Chapter 3, we did not see very much contribution of the rewarded air puff to POm activity, even when mice knew the association and expected rewards. These results, in combination with the fact that a movement other than whisking (namely, licking) contributes to POm activity, might suggest that POm is responding to body movements generally. Indeed, body movement signals have been shown to contribute a large amount to neural activity across the dorsal cortex, including in sensory regions, and even across the brain (Musall et al. 2019; Stringer et al. 2019). POm is a potential convergence zone between external sensory signals from the brainstem and motor signals from cortical regions and might be involved in the integration of sensory stimuli with movements.

### *4.2.3 Signatures of learning, or lack thereof, in POm*

Changes in the POm to S1 synapse after sensory-reward association training suggest that POm plays a role in learning within the cortex (Audette et al. 2019). Indeed, apical dendrites of layer 5 cells in S1, where the POm to S1 synapse was found to be strengthened (Audette et al.

2019), were found to have increased selectivity for task relevant air puffs (Benezra et al., submitted). We demonstrate that the movement signals within POm do not change over the course of learning sensory-reward associations. Whisking and licking continue to dominate POm activity. This result is not incompatible with a model in which POm contributes to cortical learning. Since whisking and licking happen during states of exploration or motivation (Watanabe et al. 2001; Vinck et al. 2015), POm could be more active around relevant stimuli that an animal may want to learn about. Certainly, we observed elevated POm activity on CS+ trials. POm activity could activate layer 5 cells directly and enhance the layer 5 responses to the sensory stimuli (Mease, Metz, and Groh 2016). Or, POm activity could disinhibit cortical neurons and lead to NMDAR-dependent plateaus within apical dendrites (Gambino et al. 2014; L. E. Williams and Holtmaat 2019). This activity could result in increased corticocortical plasticity and potentiation of sensory signals (Gambino et al. 2014).

## 4.3 Future directions

Many questions remain regarding the function of POm in somatosensation and behavior. Two of the major questions are whether POm encodes internal signals or movement signals and whether POm is involved in or necessary for learning about sensory stimuli. To answer these questions, the field must undertake more studies of POm activity in awake mice performing behaviors.

To determine if POm is responsive to internal, contextual signals, behavioral tasks that manipulate these signals without resulting in movements would prove useful. One contextual signal is the learned relevance of a stimulus. We designed a behavioral task in which a pole positioned in one location in the whisker field is predictive of rewards, but a physically identical pole in another location is uncorrelated with rewards (see Appendix A). This design causes one

pole to become a relevant stimulus to the animal and the other pole to be completely irrelevant. Perhaps POm would have an enhanced touch signal for the relevant pole compared to the irrelevant pole. We found, however, that mice often whisk more when presented with the relevant pole. This movement could be challenging to disentangle from a relevance-related signal.

To address the confound between movements and internal states and contexts, we need a behavioral task in which mice do not move. Amanda Kinnischtzke, a former postdoctoral fellow in our lab, considered using a fear-based task to disentangle movement and arousal. Mice freeze when presented with stimuli that cause fear (Daldrup et al. 2015). Presenting mice with a fear-inducing stimulus could cause them to freeze while also creating an internal state of high arousal. Recording activity in POm as well as body movements, such as whisking, and measures of arousal, such as pupil dilation, during this innate fear behavior would potentially allow for a way to study behavioral state modulation of POm without the confound of whisking and body movement signals.

Another option would be to attempt to train mice to suppress movement. S1-projecting axons in POm were more active during the response epoch in a behavioral task even when mice were trained to suppress licking as their "response" (LaTerra et al. 2020). This might be possible in an instrumental version of the Pavlovian air puff behavior presented in Chapter 3. After experiencing the rewarded air puff stimulus, mice would need to suppress whisking and licking in order to receive reward.

Even if these behavioral tasks are possible and movement and internal state variables could be disentangled, there are many questions to ask about POm with regard to whether it truly plays a role in learning. First, one could silence or lesion POm while training mice on the

Pavlovian air puff behavior presented in Chapter 3. Even though we showed that POm representations do not change with learning, we hypothesize that the movement or state signals in POm might create a window in which cortex learns about the sensory stimuli. Do mice need POm activity to remain intact in order to learn the sensory-reward association? Further analysis of our data on the effect of GRIN lens depth on learning, if any, might reveal an answer. Ultimately, the field has much work to do to determine the role of POm in learning about sensory stimuli.

Further work to determine the role POm plays in somatosensation and movement as well as its role in learning will one day contribute to our understanding of secondary sensory thalamic nuclei generally. Given the results of this dissertation, we propose that these nuclei may play a role in integrating movement or internal state signals with sensory information. They may also be involved in sensory exploration and learning about external objects. Revealing the role of secondary thalamic nuclei such as POm, in combination with what we know of primary thalamic nuclei like VPM, is essential to our understanding of how animals sense and perceive the external world with a combination of sense and movement.

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# Appendix A

Out of the array of objects in our environment, the brain selectively identifies and responds to those that are most important. We seek to understand this process in mice, who actively move their whiskers onto objects to localize and identify them. In whisker-mediated touch, primary somatosensory cortex, the first point of touch processing within the cerebral cortex, receives input from both a primary thalamic nucleus (the ventral posterior medial nucleus, VPM) and a secondary thalamic nucleus (the posterior medial nucleus, POm). Whereas VPM is thought to convey spatiotemporally precise information to the cortex, POm responses are poorly tuned and less well understood. We hypothesized that POm processes stimuli that are important in the current behavioral context. To test this, we developed a novel whisker-based detection task that requires mice to selectively process tactile input from one region of the whisker field. In this task, we present a "target" pole (indicative of reward) in one location and a physically identical "distractor" pole (not predictive of reward) in a different location (Figure A-1A,B). Mice successfully learned to associate the presence of the target pole with reward. They whisked more when the target was present but did not whisk more when only the distractor was present (Figure A-1C). Comparing the responses of POm during the presentation of the target pole and the distractor pole could reveal a role for POm in the integration of contextual signals with incoming somatosensory signals. We hypothesize that POm might have increased sensory responses for the target, but not the distractor pole. However, the fact that mice whisk more when the target pole is present creates a challenge for data interpretation given that we

demonstrate that whisking dominates activity within POm.

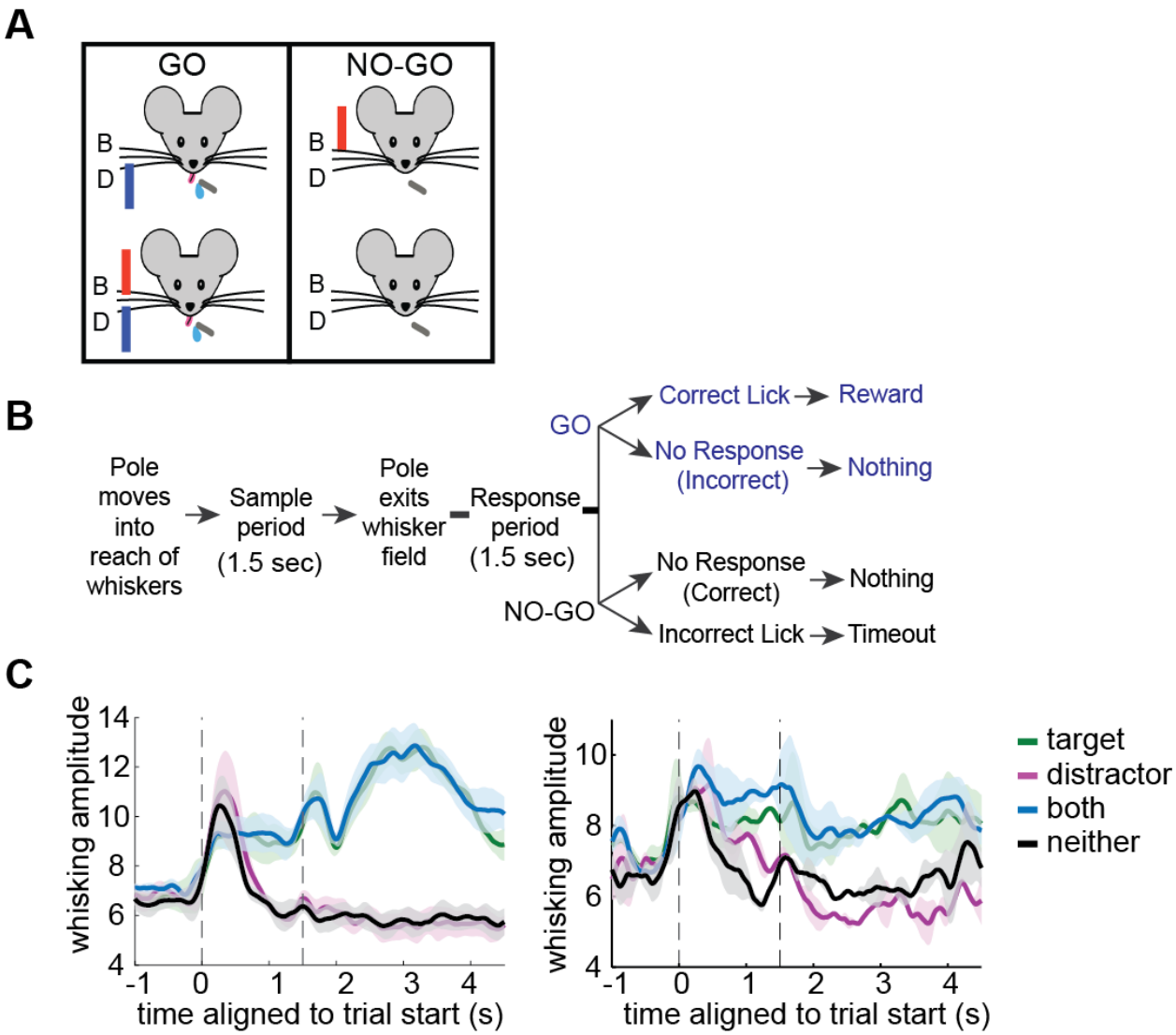


**Figure A-1 A whisker-based pole localization task with differences in behavioral relevance**
A) Schematic of the trial types of the behavior. Poles are positioned perpendicular to the whisking direction, separated by 0.5cm, and centered around the C row whiskers such that the upper whisker rows (A & B) hit the top pole and the lower rows (D & E) hit the bottom pole. One pole (in this example, the bottom pole), the "target", is correlated with rewards (behaviorally relevant to the animal) while the other pole, the "distractor" is not (behaviorally irrelevant). GO trials occur whenever the target is present and NO-GO trials occur when the target is not present. B) Flow chart of behavioral outcomes. For correct performance, mice must lick during the response period after presentation of the target pole. Licks during the sample period are not punished. C) Whisking during the behavioral task. Left, n = 4 behavioral sessions from one example mouse. Right, n = 2 behavioral sessions from another example mouse. Dotted lines indicate start and end of sample period when the pole is within reach of the whiskers. Shaded region, standard error of the mean. Target pole only trials, green; distractor only trials, magenta; both pole trials, blue; trials with neither pole presented, black.