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Trigeminal Control of Ingestive Behaviors Revealed in Prrxl1 Knockout Mice and Interrogation of Topography in the Rodent Trigeminal Pathways Using Naturalistic Volumetric Tactile Behaviors

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Abstract

All animals purposefully navigate feature-rich environments: while exploring, in search of vital resources of food and water, finding mates, and patrolling and marking habitats. During these complex behaviors, continuous analogue input information from peripheral sensory organs guides discrete and digital sequential motor output; accordingly, each action is informed, modulated, or initiated by perceptions of incoming sensory input. In turn, each action can alter the sensory stream itself, by controlling the arrangement of the sensor with the space it is sampling. This entanglement of sensory and motor spaces, creating loops and evolutionary adaptive interactions, is a fundamental function that nervous systems exquisitely display. Uncovering generalizable principles and computational processes of these interactions through neurophysiological, genetic, and behavioral studies, is one of the contemporary challenges of Neuroscience research.

However, tools and paradigms to study naturalistic complex behaviors are still lacking, with much of decades long research having been established in reductionist experiments. Even though in restricted laboratory settings, these prior crucial findings have shown remarkable adaptability and rich structures for both animal behavior and neural representations of sensory space. Two major achievements in our quest to understand nervous systems have been the uncovering of sensory and motor maps, represented in hierarchical stages of ascending neural pathways, and the discovery of higher order representations of spatial arrangement of the body in space. Mounting evidence has shown the dependance of these representations on environment context and learning, suggesting different neural computational modes when animals are engaged in more demanding and complex naturalistic behaviors. These types of experiments remain to be performed and are increasingly becoming technologically viable. The genetically tractable and behaviorally amenable rodents have been and continue to be the model of choice for behavioral and sensorimotor research. One of the aims of the work presented here is to introduce behavioral paradigms and tools to investigate complex naturalistic sensorimotor behaviors in rodents.

In particular, the rodent trigeminal modality, with its whisker system, has been uniquely useful for studying active sensing. Rodents, like mice and rats, actively sweep their vibrissae arrays and employ them in an array of ethologically important sensorimotor behaviors. These include social behavior, prey capture, purposeful haptic environment interactions, and goal-oriented exploratory behaviors, all complex behaviors that display coordination of body kinematics with the active sensor movements, besides displaying body kinematics continuously guided by whisking input. Thus, working as analogue active sensors, arranged in arrays on each side of the animal's snout, whiskers provide a rapid and modifiable mechanosensation of the proximal haptic world.

Because the topographic arrangement of whiskers on the face is mirrored in somatotopically organized nuclei in neural space, along multiple parallel ascending pathways and along the entire sensory axis from brainstem to cortex, the trigeminal system is also well suited to studying parallelization of sensory streams and functional roles for organization of sensory input into ordered and hierarchical maps. Previous and current work has been largely focused on neural representations of stimulus space in anesthetized or restrained animals, and characterization of behavioral parameters in restrained setups or trained paradigms.

In contrast, the work described here focuses on the role of trigeminal sensory information in freely moving animals, and how genetic disruption of topographical sensory organization along selective trigeminal pathways impacts natural and complex sensorimotor behaviors. We show this first in ingestive orosensory behaviors, building on pioneering earlier deafferentation studies. We confirm a crucial role for trigeminal input in eating and drinking. Our results show that trigeminal sensation modulates ingestive motor output at fast timescales. We expand these results and quantify efficiency and precision deficits across ingestive behaviors and across timescales, from milliseconds to months. This work suggests that ordered assembly of trigeminal sensory information, specifically along one of the trigeminal pathways, is critical for the rapid and precise modulation of motor circuits driving eating and drinking action sequences.

We next make use of the same genetic mouse model, in addition to wildtype animals, to lay the groundwork for investigating the role of sensory organization in naturalistic and complex volumetric

sensorimotor behaviors, focusing on whisking behaviors. This work describes an array of behavioral paradigms, a novel and flexible behavioral setup and methodology for conducting haptic exploratory behaviors where all degrees of movements are available to the animal, in full three-dimensional space. In these experiments, environment context and untrained self-initiated sensorimotor interactions can be studied. We illustrate some results on whisking kinematics and show and discuss advances on tracking whisking and animal behavior.

In summary, taken together, we hope that the work presented here will advance our understanding of the functional importance of topographic organization along specific somatosensory pathways. We also expect that the methodology and work described, and the data collected, will provide a rich repository for future studies and enable investigation of crucial sensorimotor questions. We encourage the field to push the envelope and increase the proportion of studies that focus on naturalistic complex, multimodal behaviors. Immediate future results can focus on how head and body parts orientation and movements during our volumetric haptic exploration datasets, are coordinated with the active sensing movements of whiskers. We hope that coupling of active sensory and motor spaces can be investigated, models and hypotheses can be tested, and that exciting new tools and paradigms can build on our work to investigate questions of neural and behavioral computations, including latent learning.

The rodent trigeminal system and whisking behaviors have already shown the rich complexities and adaptabilities in both behavior and neural spaces. Cracking open and modelling their complex behavioral processes and sensorimotor loops is one of the next main big and exciting challenges for Neuroscience. Our understanding of neural systems, their function, cognition, and intelligence is dependent on these advancements.

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This thesis is a journey. An almost seven years long journey I did not take alone. Even though this quest for the perfect shrubbery and the appropriate path towards it took me in unexpected, often solitary trackways into the enchanted forest of Neuroscience, at each step and each turn there were friends, colleagues, family and advisors watching and encouraging me to continue steadfast. In this regard, this may seem like a typical quest that many a graduate students embark, filled with uncertainty, often not quantifiable, with unexpected shifting tracks and routes that have changing lengths. But it was my specific journey, and, in this section, I would like to acknowledge and give thanks to all that made it happen and were there to support me and this work.

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I will conclude with a special acknowledgement to the subjects that were part of my projects and results. When describing the thesis work, I will summarize to others that I studied the brain and behavior. But it is not correct to leave it at just that and not acknowledge the subjects. I studied their brains and their behaviors. Each mouse and rat that partook in experiments has shown that individual data matters. That behavior is contextual, and a big part of that context is the individual. Learning occurs and variance is an important descriptor. The undergraduates that helped with some of the behavioral experiments were able to closely associate defined traits to individuals, and these traits were shared among many observers. One day we might gain a better handle at the hidden latent variables of behavior as an organism's neural circuits dynamically process flowing information. Until then, I can describe their behavior with traits that I know and observe in humans. I can say that the subjects in my experiments were playful, inquisitive, adaptive and skillful. And without whom this work would not have been possible.

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Chapter 1 : Introduction

1.1 Sensing to move and moving to sense: a fundamental duality of nervous systems

Since the escape from primordial soups(Haldane, 1929; Tirard, 2010, 2017; Wolos et al., 2020), animals and genes have adapted for complex and fast movements (Bennett, 1991; He & Deem, 2010; Lu et al., 2012; Meyer et al., 2021), sensing at bigger ranges (Long & Gordon, 2004; MacIver et al., 2017) and making use of adaptive strategies in complex environmental features (Mugan & MacIver, 2020; Stephens, 2007; Yoo et al., 2020). Purposeful movements are guided by specific needs like hunger, finding prey, finding mates, or avoiding predators, all while navigating locally feature-rich and globally patchy environments (Hayden et al., 2011; Thompson & Fedak, 2001).

Sensory systems aid in moment-to-moment guiding of sequences of motion that subserve these needs. Examples include navigation by phototaxis in insects (Paulk et al., 2013), chemotaxis by animals with very small neural repertoires (Bargmann & Horvitz, 1991; Hilliard et al., 2002; Troemel, 1999), whisker guided wall following and burrow navigation by rodents (Sofroniew et al., 2014; Vincent, 1912), and visually-guided flight or capture of prey (Altshuler & Srinivasan, 2018; Land, 1973; Orger et al., 2008). Primate visual systems can rapidly extract meaning of behaviorally relevant features from a visual scene. Monkeys and humans respond to a briefly flashed photograph and decide if it has either food or an animal in as little as 160-200ms (Fabre-Thorpe et al., 1998; Thorpe et al., 1996). Freely moving rats sample and discriminate between a rough and smooth surface with only one to three touches of whiskers in 100-300ms (Von Heimendahl et al., 2007).

Movement in turn, causes a spatiotemporal modification in the coupling between the sensory organ and environment, inducing changes in features of a stimuli being sensed. As animals move, the physical energies facing the sensing organ inherently fluctuate, even when the world is stationary. Sensory systems and their first neural relays are placed in the periphery of an animal. The flow of information

projected onto that neural space, as the sensed energy is transduced, is thus constantly updated with movement.

In vision, optical flow of visual features is actively used to detect higher order representations, such as stimulus speed, size, orientation, or heading speed (Orban et al., 1992; Rogers & Koenderink, 1986; Segawa et al., 2012; Warren et al., 1988). These primary feature detectors can be found as early as the retina (Enroth-Cugell & Robson, 1966; Fisher et al., 2015; Poggio & Reichardt, 1976). Small saccadic eye movements actively change gaze location in high visual acuity tasks and enhance spatial detail (Ko et al., 2010; Rucci et al., 2007).

Tracking of plumes of odorants by many animals, including mammals (Liu et al., 2020; Porter et al., 2007; Thesen et al., 1993), is another example of a behavior during which continuous sampling of a stream of information is followed by neural processing of this information, which in turn changes the next sequence of movements. The orientation and movement of the head plays a key role in this behavior, as it constrains access to the olfactory plume. Finding prey, avoiding predators, navigating the olfactory landscape, are all ethologically important sensorimotor behaviors that require following gradients of stimuli. Sensory systems performing active sampling have adapted to employ fast and efficient neural computations that are necessary for enacting quick planned changes in muscle activation sequences.

Fast neural computations of energy sources, like sound, are actively used for localization by many animals. Owls are one such model organism, used for their ability to perform three-dimensional auditory guided prey capture behaviors (Konishi, 1973). Head is positioned according to binaural signal integration, which has revealed neural representations and maps for both timing and amplitude coding schemes. These mechanisms are thought to be actively used in determining distance and angle of emitting sources (Knudsen & Konishi, 1979; Konishi, 1973). This computation occurs as early as the brainstem (Carr & Konishi, 1990), an example of neural computations that are fast and require only a couple of neural processing levels (Grothe et al., 2010). Some rodents, like bats, navigate complex volumetric 3D terrains (Finkelstein et al., 2016) by sensing their own emitted auditory source, a behavior termed echolocation. This active control of the timing and direction of the energy, helps create a spatial

map of features in the environment, including finding signatures of small prey in the reflected sensory stream (Fujioka et al., 2016).

As the flow of sensory information is continuously sampled, features that don't match the statistics of the recent encounter history or that fall outside the expected distributions can be detected. These can be as simple as unexpected branches of trees creating visual edges as the head or body turns during running. Surfaces that are different in texture or compliance from the local terrain such as cracks in the pavement, or emergence of a new odorant gradient, are all examples of high contrast changes as an animal locomotes. These high contrast changes provide a salient signal that can quickly be filtered and recognized by the underlying neural substrates, and which can be coupled to very quick reflex arcs or more complicated movement responses.

Motor systems also lie in the periphery of the organisms with muscles and their motor command neurons orchestrating the synchrony of movement syllables. In the case of reflex arcs, they are inherently tied to the sensory stream and the response can be as quick as a few milliseconds, with the entire circuit consisting of as few as two neurons as in the case of the stretch reflex (Liddell & Sherrington, 1924). As motor systems respond, the animal's position in space and thus its sensory panorama has shifted.

The new environment state informs the next steps of movement. In this mode, an organism undergoes a feedback chain of sampling environment energies and reacting to perceptual changes as they sense. How the body moves, and where the sensor is positioned, affects the next available packet of sensory information. So, while the environment informs what is sensed, moving the sensors into available stimuli space to extract more information from vantage points, is a way to change the environment space that can be sampled. Blind humans move their hands along edges of objects, haptically sampling the features that are more informative of shape and change in curvature (Lederman & Klatzky, 1987; Tramper & Flanders, 2013). Rodents actively move their vibrissae (whiskers) and modulate their amplitudes and frequencies upon encountering a texture or shape (Carvell & Simons, 1990; Grant et al., 2009; Sachdev et al., 2003). As objects have varied compliance, modulating whisking could be a mechanism for extracting more information during mechanosensory exploration (Birdwell et al., 2007).

This form of actively modulating the sensor with incoming afferent input is called active sensing. The sensor moves, often in concert with the body, or emits the energy in order to sense. Bats echolocating are an example of active sensing for three-dimensional navigation (Finkelstein et al., 2015), as is whisking behavior in rodents (Prescott et al., 2011). One is an example of distance sensing and forming a representational map of the environment, while the other is an example of haptic sensing of proximal space.

Coupling of sensing and movement forms a functional duality in neural systems. Animals sense to move and they move to sense. Sensory neurons process incoming stimuli, and the processed output informs motor programs and motor neuron populations sequence of firing. Muscle group activations changes the body kinematics, repositioning the sensor and refining and configuring the next epoch of information sampling and subsequent movements.

Purposeful exploration and navigation of an environment is a prime example behavior that employs tightly coupled sensorimotor function. Active tactile sensing is a very useful modality to study this interaction (Chiel et al., 2009; Kleinfeld et al., 2006; Nelson & MacIver, 2006; Schroeder et al., 2010). It is then not surprising that the rodent whisking behavior has been one of the main Neuroscience models of choice for studying active sensing for well over a century (Vincent, 1912).

1.2 Tactile sensing with vibrissae in rodents as an exemplary model for active sensing

“For a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied.” (Krogh, 1929) Kogh’s principle is embodied in studying active sensing via whisking behaviors of rodents (Bosman et al., 2011; Mitchinson et al., 2011). The modern-day investigational approaches include genetic dissection of neural circuits and the principle can be extended as “… there will be some animal of choice and genes of choice...” ("Krogh's principle for a new era," 2003). Ability to express effector and reporter proteins has given rise to a rich repertoire of optogenetic and chemogenetic tools (Wiegert et al., 2017). Other functional perturbations include gene deletions (knockouts) or gene mutations. This is especially useful when the genes in question affect the assembly

of neurons in an area of interest, specific neural circuits (connectivity), specific neural pathways (projections), or specific cell types. Genetic perturbations and circuit dissections via genetic tools are being used to revisit many open questions in the rodent vibrissa system.

All mammals besides humans and egg-laying mammals (monotremes) have vibrissae (Chernova, 2006; Muchlinski, 2010). A small set of mammals, such as shrews (Munz et al., 2010), gerbils, rats, mice, chinchillas and porcupines (Woolsey et al., 1975), actively move the vibrissae. Rodents protract and retract their vibrissae in a rostro-caudal fashion creating 'whisking' sweeps. These hair-like structures are embedded in neatly arranged follicles in the mystacial pad of the animals, symmetrical on both sides of the face (Ebara et al., 2002; Rice et al., 1986). This gross arrangement in rows and column fashion is preserved throughout the mammalian order, but the detailed organization varies between species (Brecht et al., 1997; Woolsey et al., 1975). This topography is observed in both animals that actively move their whiskers and ones that have evolutionary lost or never gained this ability.

There are no sensors along the shaft of these hairs that protrude out of the face. Instead, all sensing happens within the follicle complex by a number of different mechanoreceptor types (Ebara et al., 2002; Patrizi & Munger, 1966). The specific energies they sample and diversity of responses in these mechanoreceptor types to stimuli features, is an active area of ongoing investigation with models of full three-dimensional mechanical variables best capturing the response space (Bush et al., 2016). The tactile sensitivity of vibrissae has been shown to be comparable to primate fingertips (Carvell & Simons, 1990). The first neurons of the system, residing in the trigeminal ganglion, get their input from one of these receptor types (Zucker & Welker, 1969).

Each whisker follicle is connected to several muscle types. Coupling to one adjacent follicle via an intrinsic sling muscle serves as the main protraction mechanism for individual vibrissae, while extrinsic muscles connected to larger portions of the mystacial pad elicit whole array movement (Dörfl, 1985). There is row-wise actuation of whiskers as extrinsic muscles connect to an entire row, and this actuation happens on all rows at once (Berg & Kleinfeld, 2003; Hill et al., 2008; Simony et al., 2010), suggesting a coarse control of whisking via multiphase neuromuscular activity. Exploratory contactless whisking in air

tends to be in bouts, with each bout having different characteristics such as different frequency (Berg & Kleinfeld, 2003), a simple observation that makes whisking not only a good active sensing model, but also a great model for patterned dynamic motor activity.

Other muscle groups exist that connect to columns or selective rows (Haidarliu et al., 2010; Sarko et al., 2011). The synergistic activation of several of these muscle groups results in the oscillatory typical whisking of 8-12 Hz in rats (Bermejo et al., 2005). The specific patterns of activation and their timing can result in the finer subwhisking timescale of control, such as touch-induced whisking 'pumps' (Deutsch et al., 2012; Wallach et al., 2020), or fanning of whisker array spread (Grant et al., 2009; Haidarliu et al., 2010).

It is well established by now that whisker movements are finely controlled and not just an oscillatory process guided by a central pattern generator (Berg & Kleinfeld, 2003; Gao et al., 2003; Moore et al., 2013; Welker, 1964). Rodents can whisk asymmetrically, asynchronously and rapidly modulate both frequency and amplitude of whisking (Towal & Hartmann, 2006; Towal & Hartmann, 2008). Upon touch, reflexive like behaviors, such as rapid retraction, but then followed by maintenance of gentle protractions and palpations against objects are observed (Berg & Kleinfeld, 2003; Carvell & Simons, 1995; Harvey et al., 2001). When perched from a platform and sensing a gap, high amplitude whisking and maintenance of this high amplitude protraction angle seems to facilitate extensions of object and edge sensing (Berg & Kleinfeld, 2003). This behavior is observed as rodents run through a maze (Arkley et al., 2014), keeping their whiskers protracted forward, presumably to decrease the reaction time from sensing to action. Contact durations between sequential whisks when sensing a surface are modifiable (Grant et al., 2009) and the nose and head plays a coordinating role (Hobbs et al., 2016), with whisking control resembling haptic grasps. All these behaviors make rodents an excellent model for studying active sensation.

The sensorimotor coupling in rodent active whisking behaviors naturally extends to the neural space. Whisker primary somatosensory (wS1) and whisker motor cortex (wM1) are positioned adjacent to each other with dense reciprocal connectivity (Porter & White, 1983; White & DeAmicis, 1977). Recent evidence points to functionally integrated sensorimotor signals as early as wS1, with wM1 innervating

almost all layers and directly connecting to inhibitory and excitatory neurons (Kinnischtzke et al., 2014), redefining the classically named somatosensory cortex as a sensorimotor cortex (Ferezou et al., 2007). Even as early as brainstem, only three neurons comprise the shortest sensorimotor loop (Matthews et al., 2015), with the sensory afferent signal from the trigeminal ganglion relaying onto a spinal trigeminal nucleus and then onto facial nucleus motor neurons innervating the mystacial pad.

1.3 Neural sensory maps and their role in sensory processing

Vibrissae innervating trigeminal sensory neurons are pseudounipolar cells that reside in the trigeminal ganglion (Vg). Each neuron connects to only one whisker (Kerr & Lysak, 1964; Lichtenstein et al., 1990) and there is some evidence for only one mechanoreceptor type innervation from each neuron (Furuta et al., 2020; Severson et al., 2017; Waite & Jacquin, 1992). About 150-200 neurons in the rodent connect to about 7 mechanoreceptor types (Boada, 2013; Ebara et al., 2002; Rice et al., 1993). The relatively small input size makes the whisker system tractable for investigating sensory computations and modeling sensory organization from whisker follicle to cortex.

Vg neurons project diffusely throughout the sensory brainstem trigeminal complex, each innervating all nuclei simultaneously. Innervated structures include the principal trigeminal nucleus (PrV) and the spinal trigeminal nuclei (interpolaris - SpVi, oralis - SpVo, and caudalis - SpVc) (Clarke & Bowsher, 1962; Hayashi, 1980; Torvik, 1956). These bifurcations provide a unique model to study likely functionally distinct roles in branching common input, for both post-synaptic computations and pre-synaptic processing by parallel branches of the same neurons.

In contrast, other sensory modalities such as olfaction, vision and audition, have an explicit neural space separation at the source of the sensory stream. Olfactory sensory neurons also preferentially connect to only one receptor type out of a pool of about 1000 (Buck & Axel, 1991; Zhang & Firestein, 2002). But, while scattered and distributed throughout the olfactory

epithelium, same receptor type neurons coalesce their axonal terminals onto individual neuropils in the olfactory bulb, segregating that input space into separate neural processing units called glomeruli (Mombaerts et al., 1996; Soucy et al., 2009). Moreover, this separation is genetic, with each sensory neuron expressing only one olfactory receptor gene (Mombaerts, 2004) and thus receptor type. Each receptor type has affinity to a set of odorant molecules (Nagayama et al., 2014). Instead of input parallelization onto all the first processing centers displayed by the trigeminal nerve, olfaction displays an input partition.

This segregation of sensory input stream at the source, is also seen in audition and vision. Hair cells are neatly arranged along a frequency gradient on the basilar membrane creating a tonotopic map (Fettiplace & Fuchs, 1999). Rod and cone photoreceptors connect to second order bipolar cells in a columnar manner, imbuing output retinal ganglion cells with retinotopy (Roska & Werblin, 2001) which is relayed all the way to cortex (Dräger, 1975; Tusa et al., 1978). About a dozen bipolar cell types organize in diverse synaptic and microcircuit motifs (Demb & Singer, 2015; Grunert & Martin, 2020; Wassle et al., 2009), with retinal ganglion cells integrating weighted input from these parallelized streams in non-linear fashion (Duan et al., 2014; Jacoby & Schwartz, 2017; Schwartz et al., 2012). The retina itself functions like the collection of trigeminal sensory brainstem nuclei, with at least 20-30 functionally distinct output channels provided by the reported retinal ganglion cell types (Demb & Singer, 2015). This spatiofunctional organization includes temporal components with some evidence for segregated computations in different terminals/branches of the same neuron (Asari & Meister, 2012).

Early organization of sensory systems into functional units forms sensory maps. Advantages of mapped input spaces include gain control, reduction in wiring connectivity, and facilitation of local circuit lateral interactions. These topographic arrangements can enable quick filtering and

decorrelation of input at the starting stages of sensory processing (Arevian et al., 2008). Multiple maps can coexist within the same structure as can be argued for the retina output.

The genetic signals that form these maps and the consequence of perturbing these signals are a clinical important set of questions to study because they can aid in correcting functional loss when the circuits are altered through genetic mutation or disease. More generally, understanding how sensory systems are self-assembled into neural maps and how these maps impact neural computations and acuity of perception is a quintessential pre-cursor of enabling construction of in-silico replacements.

Neural sensory maps are a substrate that can enable fast neural computations and fast sensorimotor behaviors. Active sensing systems necessitate a fast sensorimotor loop. Active sensing behaviors can then be studied for testing predictions when an ordered sensory space is neurally mismapped or the map features have changed. Quantification of neural and behavioral consequences when sensory maps are lost or perturbed can lay theoretical groundwork for testing and modeling neural computations in early sensory pathways, from small circuits to hierarchical networks.

1.4 Parallel pathways of the rodent ascending trigeminal system

Ascending rodent somatosensory pathways have been anatomically studied with multiple techniques, continuously for decades (Dörfl, 1985; Jacquin et al., 2015; M. F. Jacquin et al., 1990; Ramon y Cajal & Azoulay, 1955). The mechanosensory input from the rodent vibrissae array funnels into a tractable small set of neurons in the trigeminal ganglion (Vg). While it immediately parallelizes into several sensory processing stages, its sensory map is conserved by virtue of identity at the level of Vg. Each neuron connects to one whisker and provides a spatial labeled line (Kerr & Lysak, 1964; Lichtenstein et al., 1990). The identity of Vg neuron

predicts the spatial location of sensory input and constraints the region of space proximal to the anima's snout where contact must have occurred.

The second order sensory neurons reside in multiple nuclei along the trigeminal brainstem complex. The biggest of them, the principal trigeminal nucleus (PrV), originates the lemniscal pathway. Its projection cells travel contralaterally through the medial lemniscal bundles to target dorsal medial Ventral Posteriomedial Thalamus (VPMdm) (Erzurumlu et al., 1980; Hayashi, 1980). This ascending sensory stream is then relayed from thalamus to primary somatosensory cortex (S1) granular zone, input layer IV (Deschenes et al., 2005).

Two other named somatosensory streams have also been identified. The paralemniscal pathway ascends from the rostral portion of the interpolaris nucleus of Spinal V, SpVir. It targets the posteromedial complex of the thalamus (PoM), from which multiple collateral branches terminate in S1 dysgranular zone input layer IV and layer 5a (Kim & Ebner, 1999), S2 (Carvell & Simons, 1987), and whisker motor cortex wM1 (Deschenes et al., 2005). A third recently named pathway, extralemniscal, is a smaller parallel stream that starts at the caudal portion of the interpolaris (SpVic) and terminates in the ventrolateral VPM (VPMvl), continuing to both S2 and layer Vb of S1 (El-Boustani et al., 2020; Pierret et al., 2000; Yu et al., 2006). The specific functional roles for these parallel pathways, if any exclusive function exists, have yet to be uncovered.

From sensory cortex there is heavy top-down innervation into all the spinal trigeminal nuclei. Both S1 and S2 project diffusively throughout (M. F. Jacquin et al., 1990; Killackey et al., 1989; Smith et al., 2015; Wise & Jones, 1977). These projections actively modulate neural responses in vivo (Chakrabarti & Schwarz, 2018; Furuta et al., 2010), gating input at the level of the brainstem. Because the ascending parallel pathways directly innervate higher sensory cortices (S2) and motor cortex (M1), they are a good example of how sensory streams mix with motor

circuits in neocortex, creating multi-direction functional loops (Ebbesen et al., 2017; Matyas et al., 2010).

Lateral connectivity, intra- and longer range intercortical, can provide attention signals, sharpen tuning curves, and provide feedforward and feedback inhibition for recurrent connections and activity maintenance. This lateral connectivity exists in all levels of the ascending stream (Minnery et al., 2003; Timofeeva et al., 2003), starting at brainstem trigeminal nuclei (Li et al., 1997). There, the most caudal nuclei project to the rostral principal nucleus (PrV) (Mark F. Jacquin et al., 1990), sharpening responses of individual neurons to single whiskers (Timofeeva et al., 2004). Thus, the parallel ascending pathways together with feedback and lateral connectivity provide the neural substrates to segregate and sharpen the sensory stream at the first synaptic levels of the somatosensory stream.

There also exist pathways from trigeminal nuclei to the facial motor nucleus that controls whisker pad muscles, but also a recently discovered, faster disynaptic excitatory sensory-motor loop that gets input directly from the sensory afferents (Matthews et al., 2015). Unlike PrV, SpVi contains GABAergic and glycinergic projections which innervate the facial motor nucleus (Li et al., 1997) and can provide modulation of these brainstem-based reflex arcs. Spatial (Cohen et al., 2008) or motor areas innervated by SpVi projections suggest a possible role for localization and premotor function of the paralemniscal pathway.

Like other sensory systems, the trigeminal pathways are organized and temporally guided by developmental genes. Two such genes reported in the literature are the transcription factors Prrxl1 (also known as Drg11 in rat genomic nomenclature) and Lmxb1 (Ding et al., 2003; Jacquin et al., 2008; Xiang et al., 2010). They have been shown to be required for development of normal PrV and normal axon bundle targeting for both the trigeminal system and mechanosensory afferents in the spinal cord (Chen et al., 2001; Ding et al., 2003). Genetic

perturbations along a specific pathway and their disruption of axonal projection patterns can be used to study both function of that pathway and function of sensory maps organization in general. Behavioral consequences of such genetic perturbation are the first step in assigning functional roles to the neural computations and sensory topography along a defined sensory stream.

1.5 Somatotopy in the vibrissae-barrel neuraxis

A hallmark of somatosensory systems is spatial arrangement into somatotopic maps in neural tissue. Neighboring body areas send afferents to neighboring sensory processing areas. This extends to the overall map of the body surface for all somatosensory submodalities: mechanoreception, nociception, and thermoreception. Such explicit spatial organization, where the entire body surface is topographically organized in neural space, was coined the homunculus by Penfield & Boldrey (Penfield & Boldrey, 1937); it illustrates a humanoid figure representing arrangement of skin surface topographically mapped onto the brain and scaled by the amount of brain tissue devoted to each surface.

The homunculus appearance, with big lips and huuge hands, is a consequence of the simple fact that the neural space devoted to a patch of body surface is not a simple linear transformation. Rather, it correlates with the importance (acuity of perception and behavioral relevance) and number of afferent fibers from a body part (Krubitzer, 2007). The hands and the face are highly innervated by mechanoreceptors and occupy a relatively larger area in their respective sensory cortical locations. This cortical expansion of highest sensitive areas is then depicted in a homunculus with much bigger hands, fingers and face relative to other less sensitive body parts like the torso. In rodents, one of the largest expansions of cortical representations are the whisker responsive regions, signifying the ethological significance of the whisker mechanosensory system.

Mechanosensory afferent fibers from the skin give rise to two large ascending streams. The body stream gets its input from the entire skin surface, relaying through topographically arranged ganglia along the spinal cord (the dorsal root ganglia) (Grant, 1993; Keynes & Stern, 1984; McGlone & Reilly, 2010). In contrast, facial mechanosensory input relays through the trigeminal ganglion (Zucker & Welker, 1969) via the Vth and largest cranial nerve.

Facial topography in rodents is especially conspicuous for the arrangement of whiskers on the mystacial pad (Belford & Killackey, 1979; Erzurumlu et al., 2010). Same whisker responsive sensory neurons are clustered together, and populations of neurons tuned to nearby whiskers topographically pool into adjacent cylindrical neural clusters. These neuropil-like clusters, each receiving predominant input from unique whiskers, were coined barrels in somatosensory cortex (Woolsey & Van der Loos, 1970), barreloids in thalamus (Van Der Loos, 1976), and barrelettes (Ma & Woolsey, 1984) in brainstem sensory nuclei.

Whisker responsive primary somatosensory cortex (wS1) input layer IV became henceforth known as the barrel cortex. It displays a matrix of barrels corresponding in one-to-one fashion to the rows and columns on the rodent mystacial pad. Each barrel pools input topographically directly from thalamus barreloids. Across all cortical layers, several thousand cortical neurons form a columnar organization around a barrel, creating a processing module of feedforward, lateral and feedback influences from upper cortices, including motor cortex (Jensen & Killackey, 1987; Lefort et al., 2009; Welker et al., 1988). A barrel column in wS1 reciprocally connects to a column in wM1 (Ferezou et al., 2007) with preferential layer targeting (Aronoff et al., 2010).

The septa region between barrels is the site of spatial integration of feedback and feedforward signals (Alloway et al., 2004; Furuta et al., 2009; Kim & Ebner, 1999). The vibrissa motor cortex, S1, and S2 are linked by corticocortical connections (Chakrabarti et al., 2008; Chakraibarti & Alloway, 2006; Fabri & Burton, 1991), forming dynamical loops of information processing and

providing the neural substrate for topographic integration of spatiotemporal features sensed by whiskers.

Barrel cortex somatotopy follows through from lower topographic arrangements in trigeminal sensory brainstem and thalamus. Lesions of PrV result in the somatotopic map abolished upstream (Killackey & Fleming, 1985). The functional role of somatotopic maps in behavior remains to be elucidated. Knowing which sensory features are filtered, extracted, and perceived in each of the processing stages is crucial for developing models of neural module computations along the whisker-barrel neuroaxis.

One way to study the role of barrel-like structure somatotopy (often referred to as patterning in the literature) is to genetically perturb genes like Prrxl1 and Lmxb1, which are associated with these patterns' formation. Knocking out Prrxl1 produces a lemniscal pathway whisker somatotopy ablation, from PrV to S1 (Ding et al., 2003; Jacquin et al., 2008). Crucially, somatotopy in the extralemniscal nucleus is preserved, dissociating these two parallel pathways. Studying the behavioral and neurophysiological deficits of these animal models and comparing them with wildtype animals is a way to associate functional roles with somatotopic arrangement along the lemniscal pathway, in addition to testing the role of the pathway itself.

Behaviors that can uncover functional roles include whisking kinematics characterization, texture or shape discrimination, and localization. With hypothesized separate functions of parallel pathways, such as a discriminative 'what' function for the lemniscal stream and a localizing 'where' function for the paralemniscal stream (Diamond et al., 2008), testing naturalistic task performance in these mice can suggest behavioral relevance for characterized neural responses over decades of research.

1.6 Neural responses along parallel pathways of trigeminal system

Quantification of behavioral parameters and task performance would ultimately need to be related to neural representations, tying the action space and perceptual models to neural space and computations along a trigeminal pathway. The trigeminal nerve, with its three branches, gets input from different orofacial regions. The whisker innervating nerve, the infraorbital nerve, is the most neurophysiologically characterized branch, with responses faithfully following stimulation of individual whiskers. About 150-200 primary sensory neurons are connected to each follicle (Boada, 2013; Ebara et al., 2002; Rice et al., 1993) and they respond to an individual whisker deflection (Gibson & Welker, 1983a; Zucker & Welker, 1969) with high fidelity and rates of firing.

There is certainly a division into cell types within these ~200 primary sensory neurons and ample diversity of neural responses as each neuron connects to only one receptor type (Furuta et al., 2020; Kerr & Lysak, 1964; Severson et al., 2017). The characterization of these Vg neurons has been traditionally focused on relating firing rates to whisker kinematics variables, whisker mechanical variables or properties such as adaptability of responses to ramp and hold stimuli, almost exclusively in anesthetized or head-fixed preparations (Lichtenstein et al., 1990; Shoykhet et al., 2000). Experiments that capture whisker motion and estimate mechanical variables have shown that forces and moments at the whisker follicle base are better predictors of neural responses (Bush et al., 2016; Campagner et al., 2016), with full three-dimensional mechanics characterization performing better (Bush et al., 2021).

Classification of neural responses, and by extension their neurons, traditionally employed quantifying the adaptability to stimulus onset and offset. In this coding scheme, rapidly adapting (RA) sensory neurons are connected to RA mechanoreceptors. This coding scheme was inherited by cutaneous skin mechanoreceptor classification (Johnson, 2001), where responses

of afferent fibers connected to RA receptors are phasic and rapidly change in amplitude after stimulus onset or offset. In contrast, slowly adapting neurons maintain their firing rate after stimulus presentation. Interestingly, even earlier studies pointed out that rat whisker responsive Vg neurons cover a continuum of adaptability and they do not form a simple dichotomy (Gibson & Welker, 1983b). Moreover, both RA and SA classified neurons reach the same high firing rates when contacting an object (Leiser & Moxon, 2007), with response modulated by angle and direction of deflection (Khatri et al., 2009). More naturalistic and variable stimuli also show a continuum of responses, with adaptability and angular tuning tiling the response space (Bush et al., 2021). These diverse neural responses observed at the first station of the trigeminal system encapsulate the entire tactile sensory information that the branching ascending somatosensory pathways ought to filter, compute and relay.

Centrally, at the first sensory processing centers of the trigeminal brainstem complex, the neural responses differ between nuclei. In addition to characterization along stimulus and adaptability dimensions onto which Vg neurons are typically projected, other higher order features such as receptive field (number of whiskers that evoke a response) are used to classify brainstem sensory neurons. While Vg forwards information in parallel fashion to all sensory nuclei at once (PrV, SpVi, SpVo, and SpVc), each nucleus can differentially filter, integrate and encode its output stream. The exact functional roles for each nucleus response types are yet to be uncovered, primarily due to a lack of awake behaving data in these challenging to record structures.

Most recordings in the whisker responsive nuclei (PrV and SpVi) are in anesthetized preparations. Electrophysiological experiments have shown key differences between these nuclei and their projecting neurons. VPM projecting PrV neurons have a narrow receptive field, primarily responding to individual whisker deflections, with weaker multiwhisker responses to an

adjacent whisker. PrV neurons display direction tuning and have tonic responses to a ramp and hold stimulus (Minnery et al., 2003). These properties are largely mirrored in the next thalamic processing stage, with lateral inhibition further transforming the signal (Minnery et al., 2003; Timofeeva et al., 2003). Multiwhisker observed responses in PrV and VPM are thought to be a property of brainstem internuclear connections from SpVi to PrV (Timofeeva et al., 2004).

In contrast, SpVi neurons have large dendritic arborizations and display multi-whisker receptive fields which are organized in a row-wise elliptical shape (Jacquin et al., 1989). The rostral portion of SpVi lacks somatotopic organization into barrelettes and projects to Pom, where most neurons respond to whisker motion regardless of whether contact with an object occurs (Yu et al., 2006). This response is phasic, unlike the VPM responses, and largely occurring at the initial stages of protraction. Based on these anesthetized responses, it has been proposed that the paralemniscal pathway encodes sensor motion. Whereas the finer tuning, touch responses, and somatotopy imbue the lemniscal pathway with the components needed for object identity. But, lemniscal pathway was also reported to contain phasic information (Yu et al., 2006).

More recent awake recordings in head-fixed rats confirmed during contactless whisking in air that neurons along the lemniscal pathway can indeed encode and be modulated by rhythmic whisking on a cycle-by-cycle basis (Moore et al., 2015), as observed in anesthetized state (Yu et al., 2006). This suggests that not only touch, but also self-motion signals can travel through the same lemniscal pathway. This finding is not contrary to the proposed functional role of the lemniscal pathway for object discrimination. In order to localize and subsequently identify a tactile feature, knowledge of whisker self-motion is needed. That this information may also travel through the same pathway is consistent with the proposed role of the pathway. Yet, whether the lemniscal system does indeed support object identification or discrimination, has yet to be shown in behavior experiments or in awake recordings with tactile stimuli. VPM population

coding of more complicated whisker motion, mimicking contact with textured surfaces by stimulation with a piezoelectric device, has been shown possible in anesthetized rats (Bale et al., 2015). The ability of ascending pathways to encode both touch and self-motion is perhaps not surprising as tagged Merkel cell-associated Vg neurons have recently been shown to do precisely that (Severson et al., 2017).

The feedforward sensory streams are under influence by top-down and modulatory signals. There are promiscuous descending inputs from cortical structures all along the trigeminal spinal column (M. F. Jacquin et al., 1990; Killackey et al., 1989; Smith et al., 2015; Wise & Jones, 1977) and these projections actively modulate neural responses in vivo (Chakrabarti & Schwarz, 2018; Furuta et al., 2010), gating input at the level of the brainstem. Cholinergic modulatory signals also innervate the brainstem trigeminal complex (Timofeeva et al., 2005).

Neurons in barrel cortex have recently been shown to not only represent touch (Crochet & Petersen, 2006; Hires et al., 2015), but also kinematic parameters like whisker motion, with the output of barrel cortex containing distinct populations for motion and whisker protraction angle (Cheung et al., 2020). Higher order features like surface angle are both discriminable by mice and decodable from neural populations, with tuning curves enhanced by training on a discrimination task (Kim et al., 2020). Tuning for whisker protraction phase is also prominent in S1 neurons while mice are actively whisking on surface textures (Isett & Feldman, 2020). All these recent results on higher order tactile feature discriminations and neural representations in primary somatosensory cortex build on the finding that object detection tasks do not require barrel cortex (Hong et al., 2018).

1.7 Rodent behavioral repertoire makes active use of whisking and tactile sensing

In nature, rodents move quickly through feature-rich environments. These include dense vegetations, man-made habitats and underground tunnels. As they climb, cross open spaces or

burrow they have to actively and rapidly sense any objects and features in their proximal space so that they may modify their path and avoid danger. The whisking mechanosensory stream provides an analogue signal that can aid quick reflexes without much processing needed. At the most basic function, these touch ‘edge’ detectors can guide wall following and provide the earliest alarm of unexpected encounters in blind spots.

In the laboratory, rodent whisking behavior has mainly been characterized in head-fixed preparations due to the challenge of recording whiskers optically as they rapidly move through space. Immobilization of the animal’s head has restricted characterization of whisking kinematics in a planar two-dimensional (2D) space. Some setups have pushed the experimental boundaries, with whisking touch shown to aid active localization of walls in a head-fixed virtual reality system (Sofroniew et al., 2014). Vertical poles and edges of different orientation have been used to probe localization and discrimination function through go-nogo and 2 alternative force choice (2AFC) paradigms (Brown et al., 2021; Pammer et al., 2013).

However, the earliest studies of whisking behavior are in freely moving animals. Since the first introductions of rodents as model organisms, an array of associated behavioral paradigms has been developed, which exploits sensory-motor coupling of active whisking in moving rodents. Studies of higher cognitive functions, such as localization (Vincent, 1912), have used whisking animals as the model of choice. One example behavior includes platform localization and gap crossing (Hutson & Masterton, 1986). In this task a rodent would try to perch from an elevated location and find an edge across empty space to perform a platform jump. Experiments are typically conducted in the dark so vision input may be limited.

The ability of rodents to form a representation of the environment as they navigate in two dimensional flat surfaces has been behaviorally demonstrated in maze navigation experiments (Tolman, 1948) and neural correlates for spatial location and head direction found and

characterized in several brain areas (Hafting et al., 2005; O'Keefe & Dostrovsky, 1971; Taube et al., 1990). But, the behavior of rodents is inherently embedded in a three-dimensional world, and this is especially important for rodents which climb, burrow and pitch their heads as they navigate.

As rodents interact with the environment, evidence of the spatial arrangement of objects is accumulated. This prior and environmental-context can aid in solving tasks faster (Schroeder & Ritt, 2016). There is laboratory evidence that whisking patterns during navigation are adaptable (Arkley et al., 2014), but this comes from maze-like 2D experiments that restrict the movement repertoire and degrees of freedom. Multisensory experiments and integration of multimodal sensory streams is an open area for research. Whisker touch and olfaction are the main modalities through which rodents obtain information from their proximal environment (Diamond et al., 2008) and often in the laboratory it is impossible to eliminate the olfactory cues in an experiment.

Other sensory streams play an active role as rodents explore proximal tactile features such as edges of floors, walls, shapes and textures of objects. This active process is usually in conjunction with movement of the head (Hartmann, 2001). How the vestibular inputs integrate with whisking inputs to guide environment interaction (Mitchinson et al., 2007; Towal & Hartmann, 2006) is not presently known. It is of interest to test whether head turns gate or predict subwhisking scale features, such as touch-induced pumps (Deutsch et al., 2012).

A relationship between head movements and whisking patterns, specifically asymmetric modulation of whisking has been observed and described in exploratory and goal-oriented behaviors (Towal & Hartmann, 2006). This asymmetric control of whisking can happen during object detection and in the same whisk cycle as the initial contact (Mitchinson et al., 2007). It

has been suggested that whisker and head movements are actively controlled to increase the likelihood of environmental contacts.

There is whisking gating and sequencing of behavior by central pattern generators in the rodent brainstem, namely breathing, sniffing, and vocalization. These orosensory rhythms have been characterized and shown to have specific phase relationships (Kleinfeld et al., 2014). Orofacial motor actions are chained and coupled together (Kleinfeld et al., 2014; Kumikova et al., 2017).

Naturalistic behaviors of whisking in the laboratory have shown exquisite control of whiskers by rats (Towal & Hartmann, 2006; Towal & Hartmann, 2008) which display a rich repertoire of patterns of whisking. Upon touch whiskers retract and palpate at lower amplitudes (Carvell & Simons, 1995; Harvey et al., 2001). Head-fixed mice performing an orientation discrimination task requires a full array to learn, but once learned discriminability is then possible with a single whisker (Brown et al., 2021).

One of the bigger challenges in the study of naturalistic whisking behavior is tracking individual whiskers, especially when a partial or whole array of 35 whiskers is intact. The fine, tapered vibrissae are hard to focus on, often with a portion of the tip indistinguishable from background pixels in a video frame. This challenge is increased for freely moving behaviors, where the animal head can rapidly turn or move out of field or depth of view.

Machine vision techniques have been used to track whiskers in head-fixed preparations (Clack et al., 2012) (e.g. Whisk Janelia). These solutions, while very useful when optimized, do not generalize and require specific experimental conditions. Newer solutions, based on training deep convolutional artificial neural networks, promise to make both the labeling and training aspects of experimental video data generalizable and accessible to more researchers (Mathis et al., 2018). This has especially high utility for high-speed video data, needed for the fast-moving

vibrissae. In the near future, the challenging task of vibrissae segmentation and identification, even in the face of overlapping whiskers, promises to be solved with refinement of these deep generalizing and probabilistic modeling of spatial feature segmentations (Pereira et al., 2020).

1.8 Trigeminal afference and its role in ingestive behaviors

The study of somatosensation is not restricted to whisking touch. A primal function of tactile sensing is to aid the vital sensorimotor functions of eating and drinking. Ingestive behavior and its modulation by trigeminal afference has been studied by a few deafferentation experiments in rats (Jacquin & Zeigler, 1983; Zeigler, Semba, Egger, et al., 1984). These experiments established the necessity of trigeminal afference in gating and maintaining normal eating patterns. Deafferented rats could not drink or eat for prolonged periods post-op and gradually increased intake and food responsiveness, but never returned to pre-op conditions, unlike rats that underwent sham operations.

This aphagia and adipsia phenotype (Zeigler, Semba, Egger, et al., 1984) was accompanied by a lack of bruxism and consequential malocclusions. The sensory driven initiation of bruxism is another indication that reflexive oral and perioral tactile input aids the gating of motor sequences like grinding of teeth. Their maintenance also likely requires a persistent tactile sensory stream and its sensation. Recording from facial motor nerve while stimulating the perioral upper lip area and the intraoral areas of the roof of the mouth has shown a reflex arc from tactile sensing to motor output (Zeigler, Semba, & Jacquin, 1984). There exist direct projections from trigeminal nuclei to the facial motor nucleus that controls facial muscles like the whisker pad, but also a recently discovered, faster, disynaptic excitatory sensory-motor loop that gets input directly from the sensory afferents (Matthews et al., 2015).

The deafferented model has limitations in providing any mechanistic explanation to trigeminal modulation of ingestive behaviors. It affects all the ascending parallel pathways, and it is an

irreversible preparation. The rats eventually learn to eat, possibly by employing compensatory pathways and relying on other modalities for sensory input. Adaptation of behavior, such as higher utilization of paws and inserting snout in the food source or sipping tube was reported in the deafferentation studies.

Recent genetic perturbations and mouse models that have trigeminal deficits in specific pathways have been employed to try to uncover the mechanisms of trigeminal control of ingestive behaviors. One such gene deletion, Prrxl1 (also known as Drg11 in the rat nomenclature) causes cell loss in all trigeminal sensory nuclei and trigeminal ganglion (Jacquin et al., 2008). Its deletion selectively affects somatotopy along the lemniscal pathway (Ding et al., 2003), with barreletes present at the start of the extralemniscal pathway, but genetically ablated throughout the lemniscal pathway. This Prrxl1$^{-/-}$ mutant has been implicated in ingestive behavior deficits (Bakalar et al., 2015), suggesting a possible role for trigeminal afference organization in precise control of ingestive action sequences.

The phenotype of Prrxl1 knockouts resembles that of the deafferented rats (Bakalar et al., 2015). The mice lack bruxism and develop malocclusions (Monteiro et al., 2014). However, this mouse line is very hard to maintain, and animals die prematurely, presumably due to feeding difficulties at ages prior to weaning. While they were maintained on a liquid diet, it was shown that their weights and feeding patterns were lower than controls, implicating the trigeminal system, especially lemniscal pathway in body weight and food responsiveness (Bakalar et al., 2015). A detailed temporal analysis of chronic feeding behavior and higher temporal resolution of drinking and feeding data lacks on this useful knockout line and we describe these experiments in chapter 2.

1.9 Contributions of this thesis and experiments

This thesis describes work with freely moving mice engaged in naturalistic exploratory behaviors. A genetically perturbed mouse line is used, the Prrxl1$^{-/-}$ model, together with littermate controls. As detailed in the previous sections of the introduction, this model displays sensory disorganization along the lemniscal pathway as evidenced by lack of barrel-like structures.

In chapter 2 we describe a series of experiments comparing ingestive behavior between the lemniscal somatotopy deficient Prrxl1$^{-/-}$ mouse model and littermate controls. We confirm previous deafferentation results implicating trigeminal modulation of ingestive behavior. We replicate these studies in the mouse model and extend the findings to show active trigeminal modulation in a fast orosensory behavior. We show a trigeminal mediated ingestive deficit across timescales, from milliseconds to months. The results presented suggest trigeminal sensation actively modulates ingestive action sequences, and this modulation is likely lemniscal mediated. This mouse model with genetic ablation of somatotopic organization is shown to have utility in investigating sensorimotor behaviors, with the future aim of uncovering functional role for somatotopy along the lemniscal pathway.

In chapter 3 we describe an experimental setup for naturalistic volumetric haptic exploratory behaviors. We detail a series of behavioral paradigms using this setup and describe a dataset that uses the Prrxl1$^{-/-}$ model with the aim of uncovering behavioral consequences of somatotopy ablation from the lemniscal pathway. We show feasibility of this setup and associated paradigms by presenting preliminary tracking of head and whisker kinematics. Analysis of the collected large dataset is ongoing and will be presented at future publications. This dataset includes wildtype animals and can be used to answer questions of volumetric haptic exploration, search strategies and whisking pattern behaviors under influence of head velocities in all

directions, including pitch. Detection, localization, gap crossing, platform jumping and aperture discrimination behavioral paradigms are employed and described.

The thesis concludes with a short discussion on modeling naturalistic behaviors, functional role of parallel somatosensory pathways and multi-modal sensorimotor interactions.

Chapter 2 : Impaired trigeminal control of ingestive behavior in the Prrxl1- / - mouse is associated with a lemniscal-biased orosensory deafferentation

2.1 Abstract

Although peripheral deafferentation studies have demonstrated a critical role for trigeminal afference in modulating the orosensorimotor control of eating and drinking, the central trigeminal pathways mediating that control, as well as the timescale of control, remain to be elucidated. In rodents, three ascending somatosensory pathways process and relay orofacial mechanosensory input: the lemniscal, paralemniscal, and extralemniscal. Two of these pathways (the lemniscal and extralemniscal) exhibit highly structured topographic representations of the orofacial sensory surface, as exemplified by the one-to-one somatotopic mapping between vibrissae on the animals' face and barrelettes in brainstem, barreloids in thalamus, and barrels in cortex. Here we use the Prrxl1 knockout mouse model (also known as the DRG11 knockout) to investigate ingestive behavior deficits that may be associated with disruption of the lemniscal pathway. The Prrxl1 deletion disrupts somatotopic patterning and axonal projections throughout the lemniscal pathway but spares patterning in the extralemniscal nucleus. Our data reveal an imprecise and inefficient ingestive phenotype. Analysis of drinking behavior reveals deficits on timescales of milliseconds to seconds, while analysis of eating behavior reveals deficits over an even broader range of timescales. Food acquisition and consummatory rate data showed deficits on the timescale of seconds, and body weight data suggested deficits on the scale of long-term appetitive control. We suggest that ordered assembly of trigeminal sensory information along the lemniscal pathway is critical for the rapid and precise modulation of motor circuits driving eating and drinking action sequences.

2.2 Introduction

In rodents, sensory information from the orofacial region is critical for the moment-to-moment control of two effector systems centrally involved in ingestive behavior: the whiskers and the mouth. Active sensing by the whiskers is important for appetitive behaviors, including the localization and identification of food

and water sources. Inputs from the perioral, oral and intraoral regions modulate consummatory behaviors, including the grasping, manipulation, and licking movements involved in eating and drinking.

Both sets of orofacial inputs are conveyed to the brain by the trigeminal (V) nerve, whose cell bodies reside in the V ganglion (Vg) and branch to innervate the entire brainstem trigeminal complex, including the principal and spinal trigeminal nuclei (PrV and SpV, respectively). PrV originates the lemniscal pathway, which relays through the dorsomedial portion of the ventral posteromedial thalamus (VPMdl) to terminate in layer IV of primary somatosensory cortex (S1). SpV originates two pathways: the paralemniscal, which starts in SpVir, continues to the posteromedial complex of the thalamus (PoM), and terminates in secondary somatosensory cortex (S2), vibrissal motor cortex, and layer Va of S1; and the extralemniscal, which starts in SpVic, passes through the ventrolateral regions of (VPMvl) and continues to S2 and layer Vb of S1 (El-Boustani et al., 2020; Pierret et al., 2000; Yu et al., 2006).

Cytochrome oxidase (CO) staining reveals that several of these regions (PrV, SpVic, VPM, and S1) contain distinct topographic maps reflecting the peripheral arrangement of whiskers on the face: “barrelettes” in the brainstem, “barreloids” in thalamus, and “barrels” in cortex. However, the functional role of this somatotopic patterning, if any, remains unclear (Kaas, 1997). Moreover, whatever the contribution of the whiskers to the appetitive component of ingestive behavior, they appear to make little or no contribution to intake and body weight regulation. In rats maintained under normal lab conditions, section of the infraorbital branch of the trigeminal nerve – which innervates the whiskers – has negligible effects upon these variables (Jacquin & Zeigler, 1983).

In contrast, trigeminal oral, perioral, and intraoral inputs are critical for the sensory control of eating and drinking in rodents. Deafferentation of these regions of the face is followed by a syndrome of ingestive behavior deficits including aphagia, adipsia, incisor overgrowth, impairments in the sensorimotor control of eating and drinking, and a reduction of food- or water- reinforced operant behavior. Recovered animals show a prolonged and significantly reduced responsiveness to food and water, with recovery clearly modulated by the tactile properties of the food. The reduced responsiveness is accompanied by a reduction in the level of body weight regulation to about 80% of ad lib intake (Jacquin & Zeigler, 1983).

However, because peripheral deafferentation abolishes sensory input equally to all three trigeminal central pathways (lemniscal, paralemniscal, and extralemniscal), it is of limited utility in identifying the specific trigeminal central pathway(s) associated with the ingestive impairments. One possible approach to more selectively dissociate those pathways is to use the *Prrxl1*$^{-/-}$ "knockout" (KO) model, also known as the DRG11 KO (Chen et al., 2001; Ding et al., 2003). In this mutant somatotopic patterning is normal in SpVic, the start of the extralemniscal pathway, as well as the spinal caudalis nucleus (SpVc). It is selectively absent along the entire trigeminal lemniscal pathway from PrV to cortex. However, the *Prrxl1*$^{-/-}$ mutation also reduces the number of primary sensory neurons in the Vg by 40-50%, a loss that affects the entire trigeminal brainstem complex. The effect is most pronounced in the PrV lemniscal nucleus, with ~50% neural loss, compared to only ~25% loss in the para- and extra- lemniscal nuclei of SpVi (Ding et al., 2003; Jacquin et al., 2008). In this respect, the *Prrxl1*$^{-/-}$ mutation resembles a lemniscally-biased orosensory deafferentation. We emphasize that the deficits cannot be said to be lemniscal specific: in addition to an overall reduction in trigeminal neurons there may be other biophysical and circuitry anomalies within and outside the lemniscal system. Nevertheless, it is clear that the patterning is most disrupted along the lemniscal pathway, hence our use of the term "lemniscal-biased".

The *Prrxl1*$^{-/-}$ animal exhibits many of the deficits seen in the (recovered) peripherally deafferented rat. These include reduced eating efficiency, a reduced body weight, difficulty consuming hard food, and marked incisor overgrowth (Bakalar et al., 2015; Monteiro et al., 2016; Monteiro et al., 2014), reflecting the absence of the normal pattern of bruxism seen in rats with intact orosensory input from the incisors (Wang et al., 2007). The present study was designed to examine the ingestive behavior of this mutant at both a high temporal resolution and over extended timespans, so as to obtain baseline behavioral measures for future studies. We discuss the likelihood that the observed deficits are associated with a disruption in afference specifically along the lemniscal pathway.

2.3 Methods

All methods were approved in advance by the institutional Animal Care and Use Committee (ACUC) of Northwestern University.

2.3.1 Animals

A *Prrxl*$^{+/-}$ (129/B6 background) mouse was backcrossed three generations to CD1 strain. Subjects were adult wild-type (WT, *Prrxl1*$^{+/+}$) and mutant *Prrxl1*$^{-/-}$ (KO) Both female and male mice littermates were used (WT 3 female, 5 male; KO 5 female, 3 male). Range of ages, in months, for both groups was similar (WT 2.3-7.6 starting, 13.6-22.6 ending; KO 4.6-7.6 starting, 12.9-21.1 ending). The *Prrxl1*$^{-/-}$ mice, also known as the DRG11 line in the literature (Chen et al., 2001; Ding et al., 2003; Jacquin et al., 2008), were generated in the Feinstein lab at Hunter College (Bakalar et al., 2015).

Eight WT and eight KO mice were transferred to Northwestern University from Hunter College to participate in experiments on both drinking and feeding behaviors. Mice were at least nine weeks old at the time of transfer to Northwestern and 3-20 months old during data collection. Transferred animals were housed in a reverse light cycle room, 10hr dark:14hr light, with their dark cycle starting after 8am CST. Mice from same litter were co-housed (paired) in a cage for social enrichment.

Prrxl1$^{-/-}$ animals are fragile and require special care to survive to adulthood (Monteiro et al., 2014). Animals were maintained on a standard lab chow diet, supplemented as needed with Bioserv™ Nutra-Gel Diet™, a special soft gel formula that provides supplements of both food and water. Because the *Prrxl1*$^{-/-}$ phenotype includes malocclusions, mice were assessed carefully at least three times weekly for signs of incisor overgrowth and the teeth were clipped when necessary. If mice displayed significant signs of distress (hunched posture, ruffled fur, low mobility, significant weight drop) they were temporarily removed from the experiment and given Nutra-Gel until their health was restored. Throughout the period of data gathering, body weights, animal appearances, animal locomotion, and total food eaten was recorded daily.

2.3.2 Experiments on drinking behavior

2.3.2.1 Measurement of licking behavior

Mice were water deprived to a regime of 1 mL per diem, and water ingested during testing was supplemented to provide that daily amount. Testing did not start until at least seven consecutive days of water deprivation and at least 16hrs separated each daily session. All testing was done under IR illumination, with experimenters outside the room. At the start of a session the mouse was placed in a rectangular transparent acrylic elevated enclosure, 8”L×2”W×4”H, with entry/exit access only at one end.

Trials were self-initiated by the mouse leaving the enclosure and seeking the water reward. The session was terminated if the mouse either failed to leave the enclosure or did not begin licking within 15 consecutive minutes. At the start of a session a miniature (hypodermic) stainless steel water tube, coupled to a touch sensor, was positioned 1-4 inches from the entrance of the enclosure using a remotely controlled robotic arm. It was placed such that a mouse could reach the tube with their mouth but not jump on the apparatus. In order to receive a reward, the mouse had to position its head in front of the lick tube, and lick the tube. Immediately after the first lick was detected, a single water droplet (calibrated to 3 – 5 μL) was delivered. Only one droplet was delivered per trial. All mice continued to lick for some duration after droplet delivery. Between each reward, an inter-reward interval of at least 5 seconds was imposed.

The mice were free either to lick continuously during a trial and wait until the next trial or go back to the enclosure and, after some time, initiate another trial. Mice licked approximately 10 - 30 times for each reward dispensed. The robot arm holding the water dispensing system was not moved during a trial. To ensure that the mice explored the space and to avoid simply training mice on a repetitive set of movements, the tube’s position was changed every 5-10 minutes.

Water delivery was controlled by a solenoid valve and custom Arduino program and triggered on a hardware interrupt with millisecond precision. Contact data were collected with an Arduino, and timestamps of event changes recorded. Experimenters monitored this behavior in real-time through a

video feed to verify drinking behavior.

Licking behavior was measured using a capacitive touch sensor (Sparkfun AT42QT1010). The sensor reported state changes and each interaction with the sensor is termed a “Contact-Detach switch” (CD), reflecting either onset or offset of touching the reward tube. Therefore, the present study does not specify lick onsets or offsets, but simply the occurrence of an interaction with the sensor, thus capturing the timepoints of the behavioral transitions. Fig. 2.1A is a raster plot showing all such interactions with the sensor for one mouse on one day.

2.3.2.2 Analysis of licking

The procedure for creating histograms from rasters of licking behavior is depicted in Fig. 2.1B. For each mouse on each day, CD (contact/detach) rasters were summed and then smoothed with a moving window of 51 msec (25 msec on either side of a central value). The value of 51 msec was chosen as a compromise: we aimed to avoid magnifying the variability between trials and between animals, while also not time-averaging over so long a duration that all temporal structure vanished. All histograms were normalized so the area under the curve was 1. Results for values of 11, 51, 81, 101, 201, and 1001 msec are shown in Supplementary Fig. 2.S1.

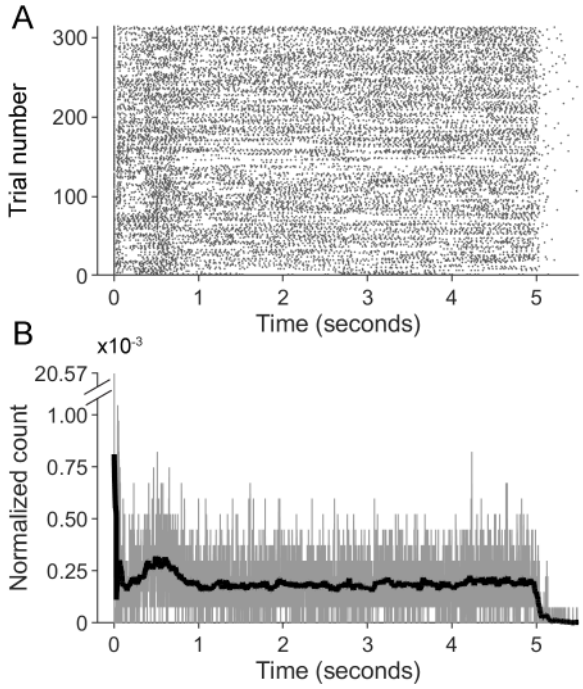


Figure 2.1 An example of measurements of sensor contact/detaches and procedure for computing histograms.
(A) An example of a raster plot of contacts and detaches (CDs) from one mouse during one session. (B) To create histograms quantifying licking behavior, rasters such as that shown in (A) were summed (black trace) and then smoothed with a window of 51 msec (light gray trace, overlaid). The trials are aligned on first lick, so the large value at zero is related to the number of trials.

2.3.3 Experiments on feeding behavior

2.3.3.1 Monitoring food intake

Body weight and total food consumed each day (Chow pellets, Nutra-Gel) were recorded for each animal by subtracting the amount remaining from the amount provided the previous day. To ensure that mice with malocclusions had equal access to hard food, 2-6 pellets of different sizes were dispensed each day both on the cage floor and on the wire lid top.

As described in earlier studies (Monteiro et al., 2016; Monteiro et al., 2014), *Prrxl1*$^{-/-}$ animals occasionally displayed health complications, such as severe malocclusions, transient *alopecia* (particularly in overgroomed caudodorsal areas), poor locomotion, hunched posture and ruffled fur, and transient weight

drops (presumably associated with insufficient consumption of hard food). During these periods, we supplemented the animals with 2-8g of soft food and/or removed them from water restriction and/or provided higher *per diem* water amounts until the symptoms resolved. All the data used in our analyses comes from animals assessed as healthy on the day of data collection.

To control for these variations in diet, the results presented in Fig. 3 include only days when animals were not receiving soft food for at least two days and were not water deprived for at least five consecutive days. Two pairs of WT animals and two pairs of KO animals were from the same litter and co-housed throughout for social enrichment. For these pair-housed individuals, we report the mean consumption per animal, that is, total amount consumed per cage divided by two. Mice were between 2 and 23 months old during the 17 months of food consumption behavior recordings.

2.3.3.2 Assessing feeding behavior

To assess feeding behavior in frame-by-frame (30 fps) video analysis (Table 2.1 and Fig. 2.4), a mouse was placed in a 8”L×2”W×4”H acrylic tunnel-shaped enclosure with one open side. The enclosure was elevated one foot above the tabletop so that as the mouse perched on the edge of the enclosure it whisked into empty space, unless an object or reward was deliberately placed within the search space. During periods of exploration the trainer placed a “treat” (a single piece of flavored, sugared cereal (Froot Loop™) on a platform or on a tube connected to a robotic arm and then left the room. The mouse had to notice the cereal piece and reach from the tunnel to obtain the cereal. Sometimes the mouse jumped over to the platform to obtain the cereal piece, and then immediately returned to the enclosure with it. Other times the mouse stretched to the platform and grabbed the cereal without leaving the enclosure. Mice always retreated well back into the enclosure to manipulate and consume the cereal piece. A detailed analysis of these “treat trials” was carried out.

Trials in which the mouse successfully obtained the cereal piece and began eating were deemed successful. In some trials, the mouse did not interact with the cereal at all, and on other trials, the mouse’s only interaction was to knock the cereal piece off the platform. There were also trials in which the mouse fell out of the enclosure while reaching for the cereal and in which the mouse abandoned its

attempt to obtain the cereal. The numbers of all trial result types were annotated and presented separately.

For each successful trial, we manually scored the times when: 1) the mouse first interacted with the cereal piece; 2) the mouse had the cereal piece in its paws and began eating; 3) the mouse took breaks from eating; and 4) the mouse stopped eating. In the duration between timepoint (2) and time point (4), we recorded the fraction of the cereal piece, rounded to the nearest 25%, every 20 seconds.

2.4 Results

2.4.1 The Prrxl1 mutation: General description

Fig. 2.2 outlines the nature of the *Prrxl1*$^{-/-}$ mutation as it affects topographic trigeminal sensory arrangement into somatotopy, throughout the ascending pathways. In the WT mouse, distinct somatotopy is observed in the lemniscal pathway (PrV, VPM, and S1), as well as in SpVic and SpVc. For the *Prrxl1*$^{-/-}$ deletion, somatotopy is eliminated in PrV, VPM, and SI cortex but remains intact in SpVic and SpVc. Somatotopy in the dorsal column nucleus-based lemniscal and cortical pathway were also found to be unaffected, thus the deficits in the trigeminal system associated with *Prrxl1*$^{-/-}$ deletion are PrV-specific (Ding et al., 2003).

Prrxl1$^{-/-}$ animals were distinguished by a hunched posture and ungroomed fur, which made them recognizable even to untrained observers. These traits were not present on all mutant animals, and only intermittently present even for those mutants that did display them. Consistent with previous reports, we also observed that the KO animals sometimes had *alopecia* or skin lesions, which appeared intermittently and resolved over time. A smaller subset of KO animals tended to vocalize frequently (within the range audible by humans) during handling.

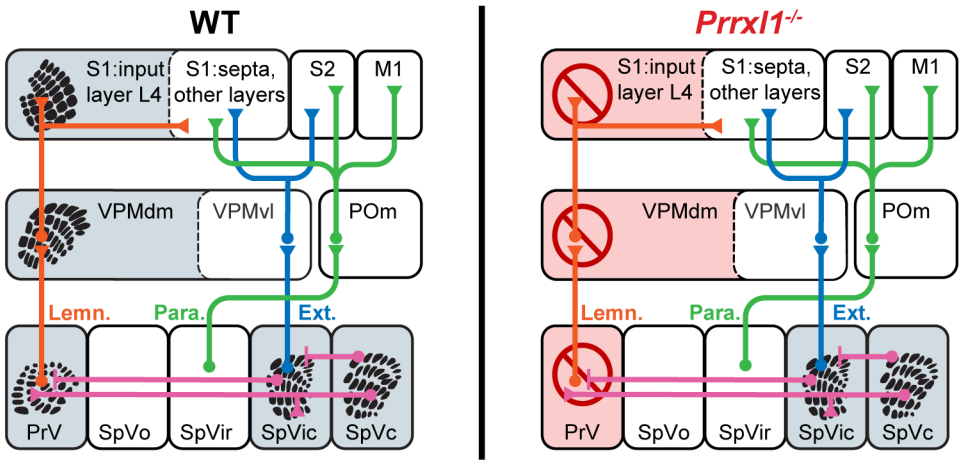


Figure 2.2 Prrxl1 deletion results in genetic ablation of somatotopy selectively along the lemniscal pathway.
Somatotopic patterning and feedforward projections of the lemniscal (Lemn., orange) paralemniscal (Para., green), and extralemniscal (Ext., blue) pathways in WT and *Prrxl1*$^{-/-}$ mice. In *Prrxl1*$^{-/-}$ mice, topographic arrangement into somatotopy is spared in SpVic and SpVc but abolished in PrV, VPM, and SI cortex - throughout the lemniscal pathway. Shaded regions mark nuclei with somatotopy present in wildtype animals. Barrel-like somatotopic patterns redrawn from (Durham & Woolsey, 1984; Meyer et al., 2013; Petersen, 2019).

2.4.2 KO mice consume less hard food and maintain lower body weights than WT mice

It was sometimes necessary to provide soft food to the KO animals to maintain their body weight and resolve transient health complications (*Methods)*. To assess the animals' ability to consume hard food pellets we selected periods when they had had *ad lib* access to water for at least 5 days and had received no soft food for the previous 2 days.

Fig. 2.3 summarizes body weight and hard food consumption for WT and KO mice for these periods (sexes and ages in *Methods*). KO mice weighed significantly less than WT mice throughout the time course of 16 months that animals were housed in our facility (Fig. 2.3A WT 42.52±7.07, PrrxI1KO 28.41±3.95; mean ± std). All but one WT mouse maintained consistently higher weight than all the KO mice (individual distributions plotted on right inset of Fig. 2.3A).

Because the weight data suggested that KO animals were eating less food, we next quantified the

amount of hard food consumed on each day (Fig. 2.3B). The weight of the hard pellets present in the cage on a given day was subtracted from the weight of the food placed in the cage on the previous day. On average, WT mice consumed slightly more hard food (WT 4.66±1.03 grams, KO 3.41± 0.32 grams; mean ± std, p=0.006 two-sided T-test). 6 out of 8 KO mice ate less, on average, than the WT mouse with the lowest average consumption. However, the distributions for KO and WT animals overlap substantially, a puzzling result given the large discrepancy in body weights. One possible explanation is that KO mice may exhibit inefficient eating behavior, such that when they bite the hard food pellets, a portion is lost on the cage floor as small fragments that could not be measured in the present experiment. An inefficient "sloppy eater" phenotype was previously observed in *Prrxl1*$^{-/-}$ animals that were reared and maintained on a liquid diet (Bakalar et al., 2015).

Although animals with lower body weight tended to consume less food, there was no clear relationship between these variables when plotted across animals (Fig. 2.3C). *Prrxl1*$^{-/-}$ animals both weigh less and eat less on average compared to WT, but this weight and food consumption relationship is not linear. Similarly, for the WT animals, for example male WT, no clear relationship exists between the variables. Interestingly, however, both groups exhibit much less variability in the weight maintained, despite large swings in daily food consumed, suggesting a homeostatic regulation of energy expenditure.

We next sought to quantify the eating efficiency of both KO and WT animals for sweet hard food (sugared cereal), which is strongly preferred by the mice.

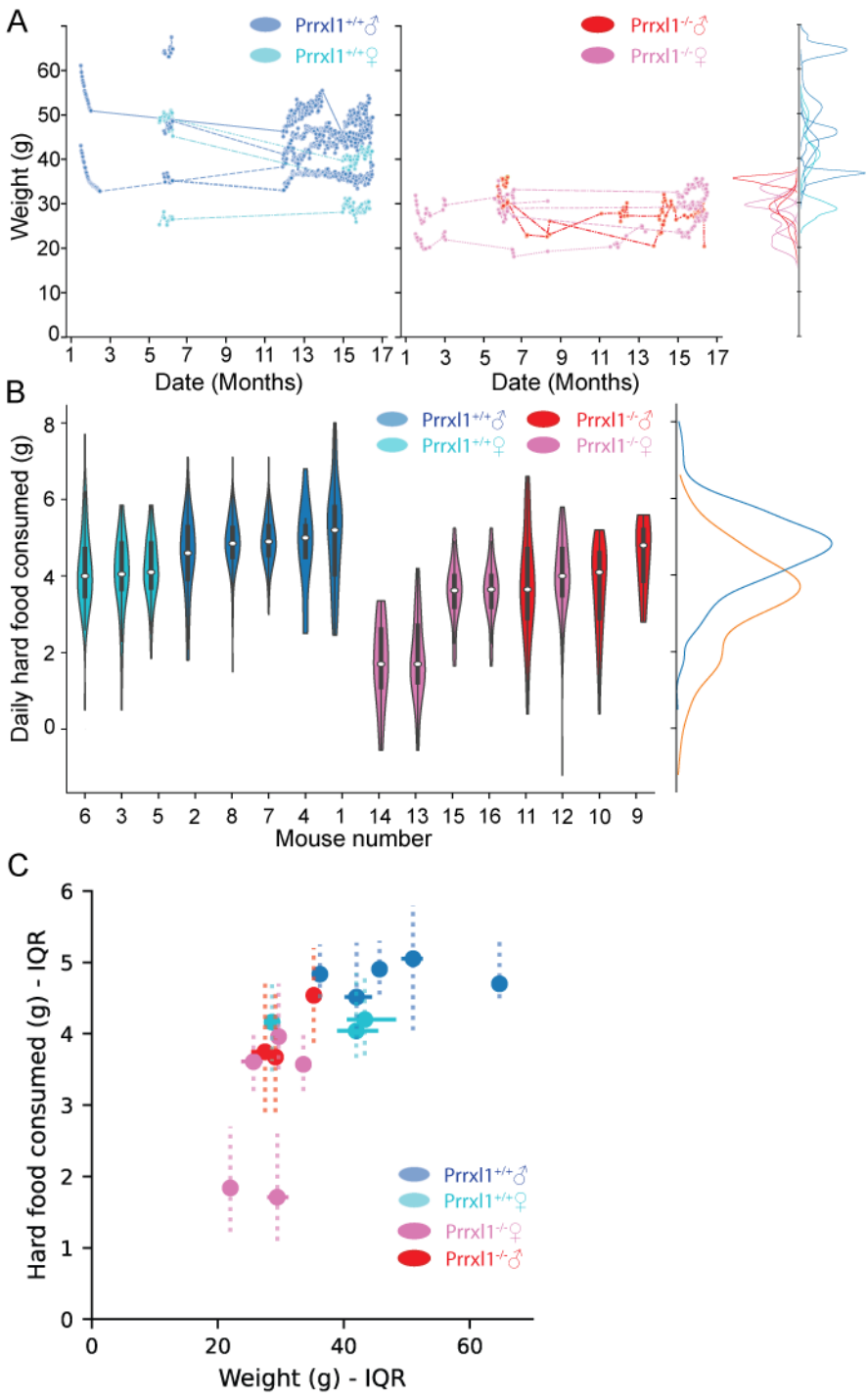


Figure 2.3 Body weights and daily food intake of WT and KO mice.
Data are shown for days of unrestricted access to hard food and water with at least 5 preceding consecutive *ad lib* water days. (A) Comparison of weights for WT and KO mice over a 17 month period. Wildtype mice (left panel, blue hues) consistently maintain higher body weights than KO mice

(right panel, red hues). Data are colored by sex in this and all subsequent panels. Only one WT animal overlaps with the KO population (individual distributions as inset on far right. WT=blue/right, KO=red/left) (B) On average, KO mice (red distributions, right side) consumed less hard food than wildtype mice (blue distributions, left side): WT 4.66±1.03 g, Prrxl1KO 3.41± 0.32 g; mean ± std, p=0.006, two-sided T-test. Six out of eight KO animals have means equal to or lower than the lowest WT. Interquartile ranges are plotted as dark bars. Population distributions plotted as inset on the far right using the same color scheme. (C) Relationship between weight and food consumed. Means and interquartile ranges are shown for both variables. None of the subgroup (e.g., male WT, dark blue) display a clear relationship between the variables. For all animals, weight is maintained within a tight range for the measured conditions (horizontal solid IQR lines), whereas food consumption varies considerably daily (vertical dotted IQR lines).

2.4.3 Prrxl1-/- mice are less efficient and less precise in their eating behavior

To measure eating efficiency, mice were presented with a single piece of sugared cereal, in a dark room, with the experimenter absent and their localization and consumption behaviors quantified. A schematic of the setup is shown in Figure 2.4A. Mice had to perch from a housing enclosure, localize the cereal placed on an elevated platform with a gap between the enclosure and the platform, and grasp the cereal with their mouth. Fig. 2.4B shows individual example video frames of the recorded behavior. Table 2.1 summarizes our findings on the behavioral variables.

The oral grasping behavior of KO mice was infrequent and inefficient compared to wildtype animals. KO animals interacted with the cereal on only 50% of the trials, compared to 75% for WT animals. On those trials in which the animals did interact with the food, all but one of WT animals (7/8; 88%) successfully grasped the cereal on nearly all (17/20; 85%) trials. In contrast, less than half of the animals (3/8; 38%) were successful in grasping. In addition, those KO animals that were successful in grasping, were successful on less than half of the trials (7/15; 47%). The remaining 5/8 KO animals either did not interact with the food (2/5 animals) or did not successfully grasp it (3/5) to start an eating session. KO mice often bumped into the cereal and knocked it off the stage or abandoned attempts to eat.

No difference between the genotypes was found in the time it took the animal to find the food, because this “discovery duration” depended on what the mouse happened to be doing at the time of its presentation. If the mouse was already at the edge of the tunnel, then it found the cereal rapidly (within 1 – 2 seconds), whereas if it was turned away from the tunnel entrance then it took much longer to find the cereal (several minutes). Similarly, no differences were observed in the number of approaches that WT

and KO mice made towards the food (usually 1-3 approaches for both genotypes). Approaches were scored as times when the mice moved with their head or body oriented towards the food, whether or not they continued to interact with the food.

We next analyzed the minute-by-minute ingestion behavior for all 7 KO trials and 15/17 WT trials with successful cereal grasping (Fig. 2.4C). Note, this analysis necessarily involves a small number of trials, since the mutants were so unsuccessful in obtaining the "treat". Two of the WT trials (mouse 7) were not analyzed because the animal knocked the cereal piece off the platform after it had started eating. Two of the three KO mice that obtained and consumed the cereal displayed eating durations more than twice as long as any of the WT mice. One of the KO mice was unable to finish more than half of the cereal, in all four trials. Only one of the three KO mouse was successful in rapidly eating the entire cereal in the two trials where they tried. In contrast, all WT mice showed fast and efficient eating behavior: on only 2/17 trials did a WT mouse stop eating the cereal part ways. KO mice eating rate in all trials for which food weight data was available (6/7 KO trials, including the two fast eating trials, 13/15 WT trials) was substantially lower than that of WT animals (Table 2.1, last row).

In summary, a detailed comparison of the ingestive behavior of WT and KO mice, at sub-second temporal resolution, indicates that *Prrxl1*$^{-/-}$ mice tend to be less efficient in oral grasping of food and modulating oromotor sequences for eating.

Behavior	**Wildtype animals**	***Prrxl1*$^{-/-}$ animals**
No interaction with cereal piece trials	7/27 trials	15/30 trials
Interaction with cereal piece trials • Knocked down cereal piece • Abandoned cereal after attempting • Fell while trying to obtain cereal • Successfully obtained cereal piece	20/27 trials 1/20 trials 1/20 trials 1/20 trials 17/20 trials	15/30 trials 5/15 trials 2/15 trials 1/15 trials 7/15 trials
Average eating rate (g/minute)	Median : 0.063 Range : 0.020- 0.132	Median : 0.012 Range : 0.005-0.069

Table 2.1 Acquisition and consumption of a piece of sugared cereal quantified for WT and Prrxl1$^{-/-}$ animals.

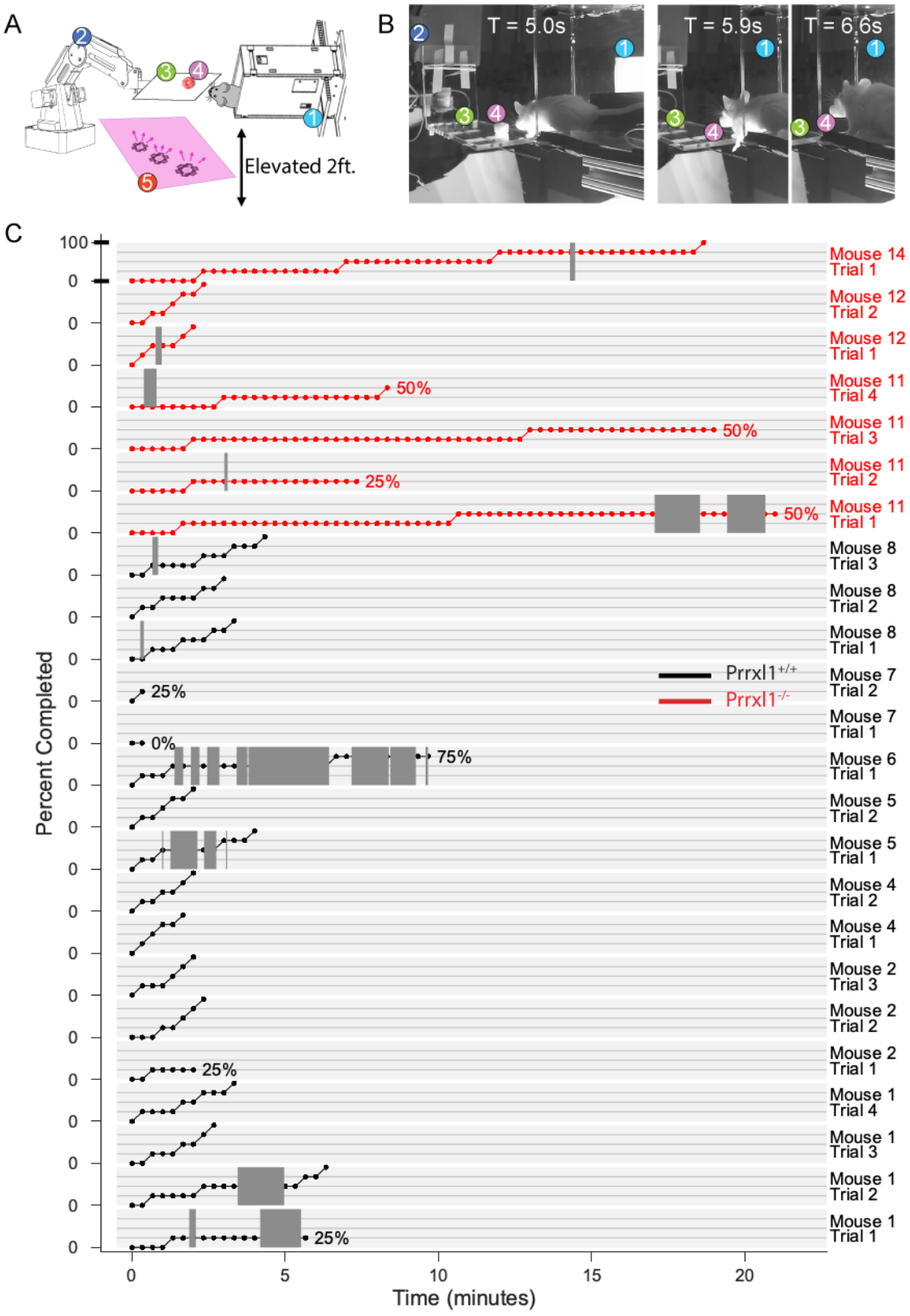

A
Elevated 2ft.
B
T = 5.0s
T = 5.9s
T = 6.6s
C
Percent Completed
Time (minutes)
Prrxl1+/+
Prrxl1-/-
Mouse 14 Trial 1
Mouse 12 Trial 2
Mouse 12 Trial 1
Mouse 11 Trial 4
Mouse 11 Trial 3
Mouse 11 Trial 2
Mouse 11 Trial 1
Mouse 8 Trial 3
Mouse 8 Trial 2
Mouse 8 Trial 1
Mouse 7 Trial 2
Mouse 7 Trial 1
Mouse 6 Trial 1
Mouse 5 Trial 2
Mouse 5 Trial 1
Mouse 4 Trial 2
Mouse 4 Trial 1
Mouse 2 Trial 3
Mouse 2 Trial 2
Mouse 2 Trial 1
Mouse 1 Trial 4
Mouse 1 Trial 3
Mouse 1 Trial 2
Mouse 1 Trial 1
50%
50%
25%
50%
25%
0%
75%
25%
25%

Figure 2.4 Consumption of a piece of sugared cereal as a function of time.
(A) A schematic of the experimental setup: (1) Mouse enclosure. (2) Remotely controlled robotic arm. (3) Clear acrylic platform for food placement. (4) Sugared treat. (5) Infrared illumination. (B) Example video frames during food acquisition. Numbers refer to the same components as in (A). Three frames capturing food acquisition behavior are shown, starting at the moment when the mouse first encounters the cereal at 5s after exploration bout initiation, and ending with the retrieval of the food treat for consumption in the safety of the enclosure at 6.6s. (C) Each light gray horizontal rectangle indicates a separate trial, with mouse number and trial number indicated on the far right. The vertical axis for each rectangle ranges between 0% and 100%, as indicated for the top plot. The 0% location is marked on the vertical axis for each trial separately. Horizontal grid lines across each rectangle indicate the 25%, 50%, and 75% levels. Data points are located every 20 seconds (dots). Within each trial, dark gray regions indicate times during which mice took breaks from eating. Plots in red show data for KO mice while plots in black show data for WT animals.

2.4.4 KO animals exhibit less consistent and less persistent licking behavior than WT animals

When water was delivered through a reward spout, both WT and KO animals licked approximately 10-30 times for each reward dispensed. Examples of typical contact-detach (CD) rasters for a WT and KO mouse are shown in Fig. 5A. In this example, the WT mouse (mouse 4) generated 69, 100, and 122 drinking trials during its first three sessions, respectively. For three equivalent sessions, a KO mouse (mouse 11, red right panel) initiated 69, 67, and 89 trials. Given that the mice had equal opportunities for licking, these data suggest a reduced responsiveness to water in the KO mouse. Inspection of Fig. 5A also suggests a reduction in the persistence of such interactions in the KO mouse, as reflected in the reduced density of the tick marks in the raster. Finally, the raster suggests higher variability in the KO behavior and less trial-to-trial consistency: later trials in each session have a lower density of events than trials earlier in a session.

While the wildtype example in Fig. 5A (left, black) shows a possible learning effect, with the event rate (density of contacts) shifting earlier for later sessions, this observation was not consistent throughout the population of WT animals, and we do not quantify any across sessions learning effects.

Fig. 5B-C generalize and quantify these results over all mice. The data plotted in Fig. 5B show that individual mice varied greatly in the number of trials generated during each session. KO mice tended to

perform significantly fewer trials than WT (mean ± std: 64 ± 36 trials/session versus 107 ± 71 trials/session; $p = 0.012$, two-sided t-test). Notably, four of the WT mice (mice 1, 6, 7, and 8) had one or more sessions in which they performed 150 trials or more, while none of the KO mice ever performed more than 145 trials per session.

Fig. 5C indicates that KO mice were also less consistent in their licking responses. The number of CDs in the first 10 trials of each session did not differ between KO and WT groups, suggesting a similar level of initial thirst. However, 5/8 KO mice generated fewer CDs in the last 10 trials of each session compared to the first 10 trials. Four of those five animals exhibited a ~67% reduction. In contrast, only two of the eight WT animals showed a reduction in the number of CDs in the last 10 trials compared to the first 10 trials. Moreover, that reduction was less severe (only ~50% of the starting trials). Taken together, results in Fig. 3B-C suggest a systemic reduction in responsiveness to water in KO animals, replicating the results of deafferentation studies.

An alternative explanation for the data of Fig. 5C is that KO mice could have increased their licking rate mid-session and then fatigued earlier, thus generating fewer total trials as well as fewer CDs at the end of the session. However, when we compare the entire distribution of CDs per trial for each animal in Fig. 5D, the two populations overlap. Moreover, the mean animal CDs per trial differ in a direction opposite to that which would be predicted from a higher mid-session licking response in KO animals (mean ± std: 23.2 ± 9.9 CD/trials for KO versus 29.4 ± 5.8 CDs/trial for WT; $p > 0.17$, two-sided t-test), with 3/8 KO mice showing a smaller mean CD/trial from any of the WT animals.

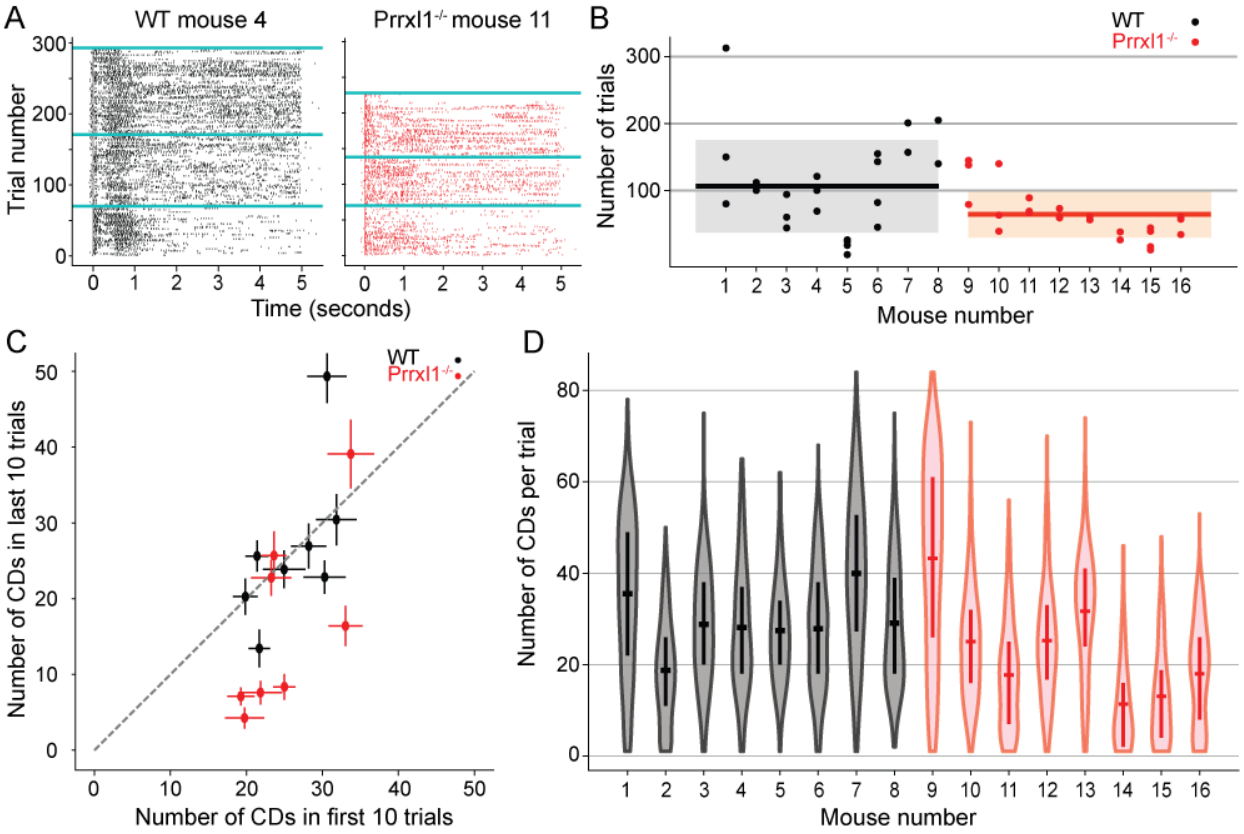


Figure 2.5 Knockout animals are less persistent and less consistent in their drinking behavior. Comparison of number of trials performed per experimental session, and number of CDs per trial for WT and KO mutant mice. (A) Typical CD raster plots WT (mouse 4, left) and KO (mouse 11, right). Each tick represents either a contact or a detach from the lick sensor. Both mice participated in three experimental sessions; sessions are divided by cyan horizontal lines. (B) WT (mice 1-8, black) tended to perform more trials than KO (mice 9-16, red). Means for both cohorts are shown as black horizontal lines, with standard deviations indicated by semi-transparent gray and red rectangles, respectively. (C) KO mice were less consistent in licking during a trial than the WT mice. Mouse numbers and colors as in (B). Licking activity for five of the eight KO mice falls well below the diagonal, while WT mice cluster close to the equality line. (D) This plot controls for the possibility that KO animals have a higher lick rate than WT and thus fatigue more quickly. KO and WT animals perform, on average, the same number of licks per trial (mean ± std: 23.2 ± 9.9 CD/trials for KO versus 29.4 ± 5.8 CDs/trial for WT; $p > 0.17$, two-sided t-test). Thus, the difference in the number of trials per session (B) and the decreased lick rate towards the end of the session (C) are not explained by a difference in effort spent per trial. Mouse numbers and colors as in (B).

2.4.5 Relation between eating and drinking behaviors

Because it is well-known that food and water intake are correlated (Possidente & Birnbaum, 1979), we

next show a comparison of the daily food consumption data and the number of lick trials (Fig. 2.6). Within a genotype, animals that performed many licking trials also tended to consume more daily food, and conversely, animals that tended to eat less daily also performed fewer lick trials. In addition, the ingestive data space suggests a functional division between the two genotypes, with Prrxl1$^{-/-}$ occupying a distinct, but partially overlapping region with wildtype mice. With the previous findings suggesting a sensory driven ingestive deficit, we next examine the temporal profiles of the licking behavior time course.

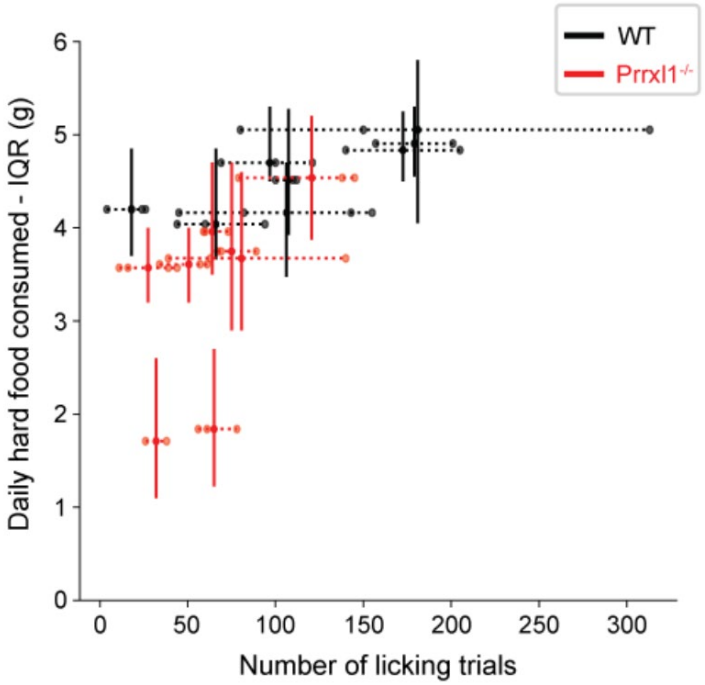


Figure 2.6. A comparison of food consumption and the number of licking trials.
Within a genotype, animals that performed more licking trials also ate more and animals that ate less tended to perform fewer licking trials. Two of the eight mutant animals (#13 and #14) display more severe food intake issues. Both groups occupy separate regions that partially overlap, suggesting two functionally distinct group in this projection of the ingestive behavior space.

2.4.6 WT and KO mice differ significantly in the time course of licking and ability to modulate licking rate

Fig. 2.7 compares the time course of licking for WT and KO mice populations as reflected in histograms

generated from the raster plots (see *Methods*). WT mice (Fig. 2.7A, black/grey traces) exhibited a pronounced modulation of licking rate upon sensory contact. This change in the lick rate starts a few milliseconds after first contact, when water delivery occurs, and peaks 100-1000 msec after reward delivery. The population histogram for all KO mice (Fig. 2.7B) displays considerably more variability, with some trials showing a decrease in licking between 100-1000 msec, followed by its resumption near 1000 msec. The standard deviation of the CD histogram shown in Fig. 2.7B is particularly broad between 100-1000 msec, suggesting that after their initial contact with the water tube, KO mice are differentially delayed at initiating repetitive licking motions, and there is larger inter-trial or inter-animal variability in this consummatory time window. In addition, the variability appears bimodal and symmetric, so that the mean of the KO CD histogram is essentially flat. The inset to panel 2.7B overlays the mean liking rates for WT and KO animals, revealing one of the strongest differences in orosensation observed.

To more closely examine the bimodal variability across the KO mice, Fig. 2.7C shows the time course of licking behavior for each mouse in order to assess the difference and consistency in the amount of lick modulation between the two groups. All WT mice modulate their licking response, precisely and rapidly upon sensory contact. In contrast, only two out of the eight KO mice (animals 9 and 13) show modest modulation of their licking behavior (8/8 WT versus 2/8 KO; $p = 0.007$, Fisher's exact test). The modulation is significantly delayed, starting after 500ms and peaking around 1s after water reward onset.

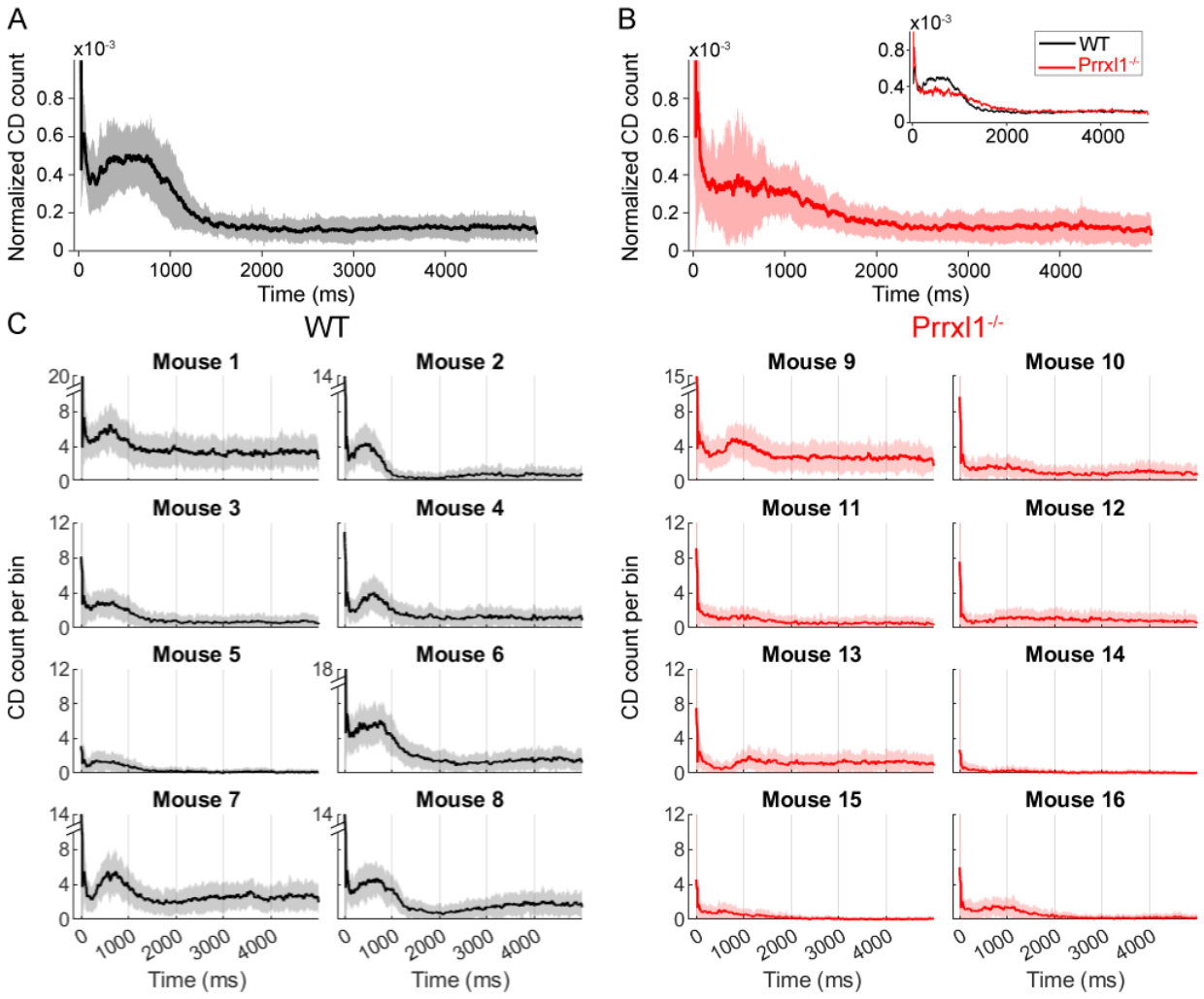


Figure 2.7 Time course of licking in WT (black and gray) and KO (red and pink) mice.
(A) All wildtype mice showed a peak in licking behavior between 1-1000 msec after the water reward was dispensed. The black curve shows the mean of the CD histogram computed across all sessions and all days for all wildtype mice. Plus, and minus standard deviation around the mean is shaded gray. (B) *Prrxl1*$^{-/-}$ mice showed much more variable licking behavior. The red curve shows the mean of the CD histogram computed across all sessions and all days for all *Prrxl1*$^{-/-}$ mice, with plus and minus standard deviation around the mean shaded light red. The *inset* overlays the mean CD counts for WT and KO animals (C) All individual mice CD histograms with a bin size of 51ms. All y-axes go to 12 CD counts/bin unless labeled with an axis break, in which case the maximum y-value is labeled.

2.5 Discussion

In the present study we examine food and water intake and the temporal organization of eating and drinking movements in the Prrxl1$^{-/-}$ mutant mouse. With appropriate husbandry (Monteiro et al., 2014), this mutant can initiate drinking, eat hard food, and live an extended life. This is an important consideration in view of its unique contribution as a model system for the study of such problems as pain, ingestive behaviors, and the origin and significance of somatotopic patterning in the mammalian somatosensory system.

2.5.1 Trigeminal central pathways and the control of feeding behavior in rodents

Deafferentation studies (e.g., (Jacquin & Zeigler, 1983), reviewed in (Zeigler et al., 1985)) have clearly identified trigeminal afference as a key source of the peripheral feedback signals driving ingestive behavior in rodents. The data in the present study were obtained in a preparation quite different from deafferented rats, and also quite different from the *Prrxl1*$^{-/-}$ KO described in (Bakalar et al., 2015). In neither of these earlier studies could the animals generate the ingestive behaviors required to maintain themselves on hard food. In contrast, the present mice, through a combination of improved husbandry and outbreeding to the CD1 mouse strain, could generate well-organized ingestive behavior sequences. In this respect, they resemble recovered deafferented animals, who could sustain themselves on hard food (Jacquin & Zeigler, 1983). The behavior of both the deafferented rats and the *Prrxl1*$^{-/-}$ mice reflects an adjustment to chronic afferent disruption, but on two different time scales: short-term, during recovery, for the deafferented rats; long-term, from birth, in the mutant. The *Prrxl1*$^{-/-}$ mutant thus allows the study of ingestive behavior in an animal whose ingestive motor sequences are present, but whose trigeminal central pathways are genetically perturbed throughout its life.

The present work shows that *Prrxl1*$^{-/-}$ animals can perch from an enclosure and use their tactile senses in complete darkness to find and subsequently consume water from a water spout whose position in space was randomly varied over time. They could also lick this spout to initiate a water reward. They did not differ from WT in the amount of licking elicited by a water reward. Importantly, they were able to ingest hard food and were viable, though at significantly reduced body weights than WT mice. Where they most

resembled the deafferented rats or early mutant mice was in the reduced efficiency of their ingestive behavior. This was most dramatically shown in the increase in unsuccessful grasps and in the increased time taken to consume a given unit of food.

2.5.2 Trigeminal modulation of oromotor sequences operates across several timescales

Eating and drinking are guided by a continuous stream of sensory information. While olfaction and vision provide distance information for localizing nutrient sources, successful ingestion depends upon a continuous assessment of the texture, hardness, temperature, and other mechanosensory properties of a food which guide the initial grasping and subsequent intraoral manipulation of the food source. All these behaviors are mediated by the jaw motor system (which is intact in the mutant) so that their impairment suggests a break in the flow of orosensory, primarily trigeminal, mechanosensory afference which elicits and modulates licking, grasping, chewing, and intraoral manipulation behaviors. This disruption operates over multiple timescales.

On a reflex timescale, an earlier study (Zeigler, Semba, & Jacquin, 1984) showed (1) that the jaw opening elicited by perioral contact of the face with a food pellet or sipper tube in the normal rat was either abolished or significantly delayed in peri-orally deafferented rats; (2) that mechanical or electrical stimulation of the orofacial region elicited reflex activity in the motoneurons of the jaw-opener muscle; and, (3) that the most effective sites for eliciting activity in the jaw-openers appeared to cluster about the region of the upper lip and superior portions of the oral cavity. Indeed, for this region, mechanical displacement of less than 1 g with a Von Frey hair was often sufficient to elicit jaw motoneuron activity in these anesthetized preparations. These observations suggest that oral and perioral regions originate the afferent component of trigeminal sensorimotor circuitry that monitors the presence and location of food and water sources and that provides continuous, moment-to moment-feedback during normal licking, grasping and intraoral manipulation. Furthermore, input from the teeth also generates the bruxism which, in normal rodents, prevents the development of malocclusion--a defining phenotype of the *Prrxl1*$^{-/-}$ mutant.

The role of trigeminal afference in modulating either the initiation of, or ongoing, ingestive behaviors

extends to longer chronic timescales in both “recovered” deafferented and *Prrxl1*$^{-/-}$ preparations. The reduction in weight and consumption rates reflects an adaptive adjustment to chronically reduced sensory input, and confirms the contribution of trigeminal afference in modulating responsiveness to food (Jacquin & Zeigler, 1983). These results link trigeminal orosensation to internal states of hunger and appetitive control (Zeigler, 1994); e.g., hedonic salience (Berridge & Kringelbach, 2008).

We emphasize that in the present study, analysis of the drinking experiments reveals a deficit in the fine control of orofacial motor activity, on the timescale of milliseconds. In contrast, analysis of the eating experiments is less conclusive. The deficits observed during eating could result from lack of interest in food or the fact that KO animals have smaller mouths. Regardless, the present work describes deficits observed at multiple spatiotemporal scales: from very fine modulation of motor circuits implicated by the drinking data, to potential changes in motivation and the consequences of altered body morphology.

The effects of reduced trigeminal afference on the modulation of ongoing oromotor behaviors is especially striking at intermediate timescales, on the order of 100’s of ms. Indeed, the striking contrast between the precise and rapid modulation of licking rates observed in the wildtype animals and its delayed or complete absence in *Prrxl1*$^{-/-}$ suggests a disrupted sensory-motor loop connecting trigeminal inputs to oromotor circuits. The timing of this behavior, which is delayed by hundreds of milliseconds in the mutant, suggests that in the mutants we are not dealing simply with the effects at a reflex level but with the involvement of higher order circuits. There is substantial top-down innervation of trigeminal sensory nuclei (M. F. Jacquin et al., 1990; Killackey et al., 1989; Smith et al., 2015; Wise & Jones, 1977), and this input modulates *in vivo* neural responses (Chakrabarti & Schwarz, 2018; Furuta et al., 2010). In addition, decorticate preparations have suggested a modulatory role for higher order structures in ingestive behaviors (Whishaw et al., 1981). The relative contributions of cortical vs brainstem structures to the trigeminal control of ingestive behaviors are important questions for future research in this mutant.

2.5.3 A hypothesized selective role for the lemniscal pathway modulating ongoing ingestive behavior

The impairments described in this report have two possible explanations. First, they could result solely from the overall reduced cell number in Vg, SpVi, and PrV, or from other cellular and biophysical deficits which may as yet be unidentified. The reduced cell number would in turn reduce signal fidelity in any or all these structures. Alternatively, the ingestive impairments could be a result of a selective disruption in trigeminal afference along the lemniscal pathway.

Support for this hypothesis comes from the study by Ding, et al (Ding et al., 2003) of the impact of Prrxl1 deletion on the lemniscal pathway. First, *Prrxl1* is not expressed in SpVi (Ding et al., 2003; Rebelo et al., 2007) or in lamina III or IV or SpVc, where barrelettes develop. It is expressed in PrV (where barrelettes are abolished with the gene deletion) and lamina I and II of SpVc (layers that don't include barrelettes). Correspondingly, barrelette patterning is normal in SpVic (the extralemniscal brainstem nucleus) and SpVc but absent in PrV (the lemniscal nucleus). Second, the absence of this patterning is associated with a reorganized axonal projection pattern at multiple stages along the lemniscal pathway. Not only do afferents to PrV fail to organize into clear whisker-specific clusters, but thalamic inputs to layer IV S1 cortex distribute uniformly instead of organizing into barrel-sized clusters as observed in WT animals (Ding et al., 2003). Lesion studies have shown that the origin of this sensory map disorganization throughout the lemniscal pathway must lie in PrV itself, and not in thalamus or cortex, where *Prrxl1* is not expressed (Killackey & Fleming, 1985).

Given the well-known feedback projections from S1 to PrV (Killackey et al., 1989; Smith et al., 2015; Wise & Jones, 1977), disorganization of these thalamocortical inputs seems likely to contribute to the behavioral disruptions at the intermediate timescale. Disordered sensory organization may cause temporally-jittered, or noisy, flow of trigeminal information leading to the impaired modulation of ingestive behavior. A selective role for the lemniscal pathway is also suggested by the observation that the ingestive impairments of the *Prrxl1*$^{-/-}$ animals included not only problems with the oral grasping manipulation of food objects, but with their initial localization (Table 1), suggesting some disruption in

vibrissal sensory localization function. The extent to which altered circuit properties and temporal jitter affect the *Prrxl1*$^{-/-}$ mutant will require electrophysiological studies comparing the response properties of SpVi and PrV neurons in wildtype and mutant animals.

Prrxl1 is also expressed in the geniculate ganglion (GG) (Rebelo et al., 2007). This sensory ganglion receives gustatory information from the anterior two-thirds of the tongue, as well as mechanosensory input from the outer ear (pinna). *Prrxl1* expression in the GG thus raises the possibility that gustatory afferents might be affected by its deletion. However, a recent transcriptomic study showed that the neurons in GG that express Prrxl1 are those that receive mechanosensory input from the pinna (Dvoryanchikov et al., 2017). This result rules out disruptions in lingual afference or gustation as the main mechanisms for the observed deficits.

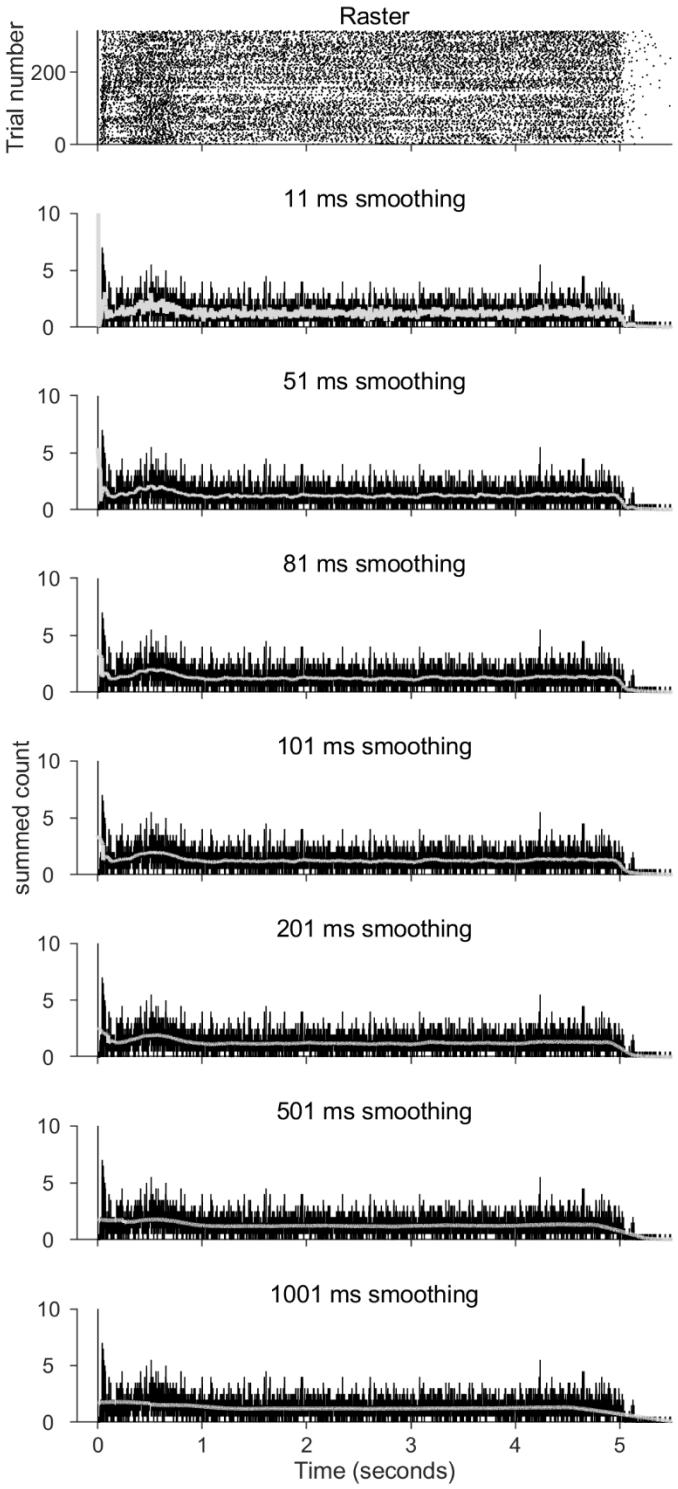
This study cannot rule out the possibility that the observed ingestive deficits result from biophysical and circuitry anomalies that lie outside the lemniscal system. Nevertheless, it is clear that functional anatomy is most disrupted along the lemniscal pathway, hence our use of the term “lemniscal-biased.” Notably, the deficits are not attributable to a specific loss of motor output. For example, during drinking behavior, WT and KO animals exhibit similar lick distributions, and they also lick at approximately the same rates when highly motivated (the first 10 trials of each day). During eating behavior, at least one animal, on one trial, was able to eat at the same rate as a WT animal. Thus, the gene ablation is an important partial functional knockout, allowing observation of graded behavioral responses. Our data are consistent with deafferentation studies which left motor nerves intact but globally abolished sensory input. Moreover, they are consistent with prior studies that have described sensory anatomical deficits in this mouse model (Chen et al., 2001; Wang et al., 2007). The extent to which finer motor output components may contribute to this model’s ingestive deficits, can be the subject of future anatomical and physiological studies.

Recent findings on vibrissal tactile sensing have suggested that the lemniscal pathway may make a unique contribution to sensory coding in the trigeminal system. Yu Et al. (Yu et al., 2006) and Moore Et al. (Moore et al., 2015) showed that neurons in the trigeminal lemniscal, but not the paralemniscal pathway are “substantially modulated” by both touch and self-motion, a coding property which is likely to be critical

for whisker-mediated discrimination. Chakrabarti and Schwarz (Chakrabarti & Schwarz, 2018) recorded from both PrV and SpVi neurons to show that sensory gating during an active whisking task affects the lemniscal, but not the extralemniscal, processing stream, and that modulatory input comes from sensorimotor cortex.

In summary, our results indicate that the ordered assembly of trigeminal sensory information is critical for the rapid and precise modulation of motor circuits driving ingestive action sequences. This trigeminal modulation is observed at multiple timescales, from milliseconds, to minutes, to months, tightly linking somatosensation and ingestion, from moment-to-moment consummatory to long term appetitive control. Our data also suggest that the lemniscal component of the ascending trigeminal pathway makes a significant contribution to that process.

2.6 Supporting information



Supplementary Figure 2.S1 Licking contacts and detaches (CDs) shown for different smoothing windows.
An example of a raster plot of contacts and detaches (CDs) from one mouse during one session shown in top panel. Effect of smoothing the summed histogram from the raster data on the top panel shown for window sizes of 11, 51, 81, 101, 201, and 1001 ms in respectively labeled panels. Black traces: summed raster data in 1ms bins, same for all smoothed histograms and overlaid for comparison. Light gray traces: the temporally smoothed average. Based on these temporal averaged data, 51ms time window chosen as the window size that preserves the temporal profile and averages out the intertrial noise. The trials are aligned on first contact, so the large value at zero is related to the number of trials.

Chapter 3 : Whisking-head coordination in naturalistic volumetric behaviors and behavioral interrogation of trigeminal sensory disorganization in the lemniscal pathway

3.1 Introduction

This chapter describes a novel behavioral setup and methodology for recording volumetric haptic exploratory behaviors that probe whisking-head coordination and haptic environment interactions in freely moving mice. We have collected a large dataset of naturalistic whisking and exploratory behaviors recorded in both wildtype and Prrxl1$^{-/-}$ mouse models (see Chapter 2 for detailed description of this model). At the time of the writing of this thesis, analyses for this dataset are still ongoing and results will appear in future publications. Here, I present the motivation for the work in the introduction, followed by methodology, describe the dataset and analyses that can exploit this dataset, preliminary results of whisking kinematics, and conclude with discussion on importance of obtaining these unbiased, untrained datasets as a means for investigating cross-modal and active sensing behaviors.

The functional neural organization of rodent whisker somatosensory ascending streams is characterized by two hallmark features: parallelization and somatotopy (Fig 3.1). The primary sensory neurons of the system reside in the trigeminal ganglion (Zucker & Welker, 1969) and bifurcate to send parallelized input simultaneously to all trigeminal sensory brainstem nuclei (Clarke & Bowsher, 1962; Hayashi, 1980; Torvik, 1956), intermixing and even combining multiple mechanoreceptor types (Sakurai et al., 2013).

This common input gives rise to at least three named parallel ascending steams: 1) Lemniscal pathway originating in the principle trigeminal nucleus (PrV), which relays through the dorsomedial portion of the ventral posteromedial thalamus (VPMdl) to terminate in layer IV of primary somatosensory cortex (S1); 2) Paralemniscal pathway originating in spinal trigeminal nucleus interporlaris, rostral subdivision (SpVir), which relays through the posteromedial complex of the thalamus (PoM) to secondary somatosensory cortex (S2), vibrissal motor cortex (wM1), and layer Va of S1; 3) Extralemniscal pathway originating in the SpVi caudal subdivision (SpVic), which relays through VPM ventrolateral region (VPMvl) to S2 and layer Vb of S1 (El-Boustani et al., 2020; Pierret et al., 2000; Yu et al., 2006).

The second feature, somatotopic anatomy, where nearby neural areas receive information from nearby whiskers, organizes sensory information topographically into cylindrically shaped neural clusters, termed barrels in somatosensory cortex (Woolsey & Van der Loos, 1970), barreloids in thalamus (Van Der Loos, 1976) and barrelettes (Ma & Woolsey, 1984) in trigeminal brainstem nuclei. This topography is observed via cytochrome oxidase staining and other labeling methods throughout the lemniscal pathway and in the extralemniscal brainstem nucleus, but not in the paralemniscal nucleus.

While topographic sensory organization in other modalities, such as retinotopy in vision (Dräger, 1975; Tusa et al., 1978), has been well studied and functional roles like ocular dominance (Hubel & Wiesel, 1977; Kremkow et al., 2016; LeVay et al., 1975; Najafian et al., 2019) and visual stimulus orientation columns (Bonhoeffer & Grinvald, 1991; Cossell et al., 2015; Hubel & Wiesel, 1963; Kremkow et al., 2016; Ohki et al., 2006) have been found in neural representations, the exact function of somotatopic maps of the ascending trigeminal parallel pathways remains a mystery. Moreover, it has not yet been established whether these maps have unique behavioral relevance in different pathways, and whether disruption of sensory organization along a somatosensory stream causes specific behavioral deficits.

Similar to sightless humans haptically exploring surface contours and changes in curvature (Lederman & Klatzky, 1987; Tramper & Flanders, 2013), freely moving rodents performing whisker localization tasks integrate over both temporally related and spatially related sensory inputs (Horev et al., 2011). Progressive entanglement of the movement signals with the afferent and re-afferent whisker sensory streams converges a motor-sensory-motor process into the perception of object location required to solve a localization task. The recently characterized contact-free asynchronous and asymmetric bilateral movement of the whisker arrays (Mitchinson et al., 2007; Towal & Hartmann, 2006; Towal et al., 2012) functionally resembles human bimanual haptic tracking (Rosenbaum et al., 2006). In addition, haptic exploration tasks have shown that rats modulate the contact duration with an object overtime, display modification of whisking patterns termed touch-induced pumps (Deutsch et al., 2012), vary array spread by significantly decreasing it during unexpected surface exploration (Grant et al., 2009) and are able to maintain whisking velocity under windy conditions in an object localization task (Saraf-Sinik et al., 2015).

This exquisite control of whiskers in rodents is coordinated with head movements (Towal & Hartmann, 2006), modulated by sensory input (Grant et al., 2009; Mitchinson et al., 2007), and is under modification by environment context (Arkley et al., 2014). These studies in planar environments in freely moving rats are supported by classical conditioning experiments in head-fixed rats which have shown ability of rats to learn to vary basic kinematic parameters of whisking, such as amplitude and frequency in a stimulus-dependent manner (Bermejo et al., 1996; Gao et al., 2003), strongly suggesting voluntary control of this active sensing behavior.

However, there is a lack of data and studies that can characterize complete three-dimensional, more naturalistic, head-movement and whisking behaviors. These ethologically important experiments are desirable given that rodent whiskers protrude from the mystacial pad forming a three-dimensional space and this embedding creates a dynamic search volume around the face of the animal (Huet & Hartmann, 2014). Whiskers are preferentially used over vision in a platform jumping task when the platform is at a different vertical placement (Schiffman et al., 1970). Rats make frequent and extensive head pitching movements and head movement and orientation dictates eye movements to maintain an overhead binocular field (Wallace et al., 2013), leaving the whiskers as the sole rapid proximal detectors of immediate space underneath and beside their heads during volumetric searches. The significance of head pitch and head-whisker coordination in the climbing and burrowing of multilayered environments during active tactile exploration and habitat navigation of rodents (Finkelstein et al., 2016) is an important open question.

In addition, findings in freely moving rats have not yet been investigated or replicated in the mouse model, partially due to the difficulty in tracking mouse whisking behavior. There is significant differences between rats and mice in the whisking kinematic variables, with mice whisking more than twice the frequency of rats (Jin et al., 2004). With recent advances in mouse large scale neural recordings and psychophysics experiments in head-fixed animals that have shown surface, shape, localization and object orientation discriminations (Brown et al., 2021; Hires et al., 2015; Kim et al., 2020; Rodgers et al., 2021), there is a

need to replicate freely moving rat findings, and compare behavior between both head-fixed and freely moving conditions as well as between species and perceptual models driving behavior.

With the help of recent advances in machine learning and deep neural networks enabled body parts and pose estimations (DeepLabCut, (Mathis et al., 2018)), we aim to track and characterize freely moving behaviors of mice performing a series of naturalistic, self-initiated tasks embedded in three-dimensions. These freely moving behaviors answer questions about behavioral strategies and head-whisker coordination during haptic volumetric search. Paradigms described include voluntary, naturalistic, self-initiated object detection, object localization, gap crossing, wall and vertical entrance localization, and shape habituation/dishabituation. Comparison between wildtype and Prrxl1$^{-/-}$ lemniscal sensory disorganization mouse models in this dataset will attribute behaviorally relevant functions to sensory topography along the lemniscal pathway and put bounds on time parameters for behavior which can aid in modeling underlying neural circuits topology and computations.

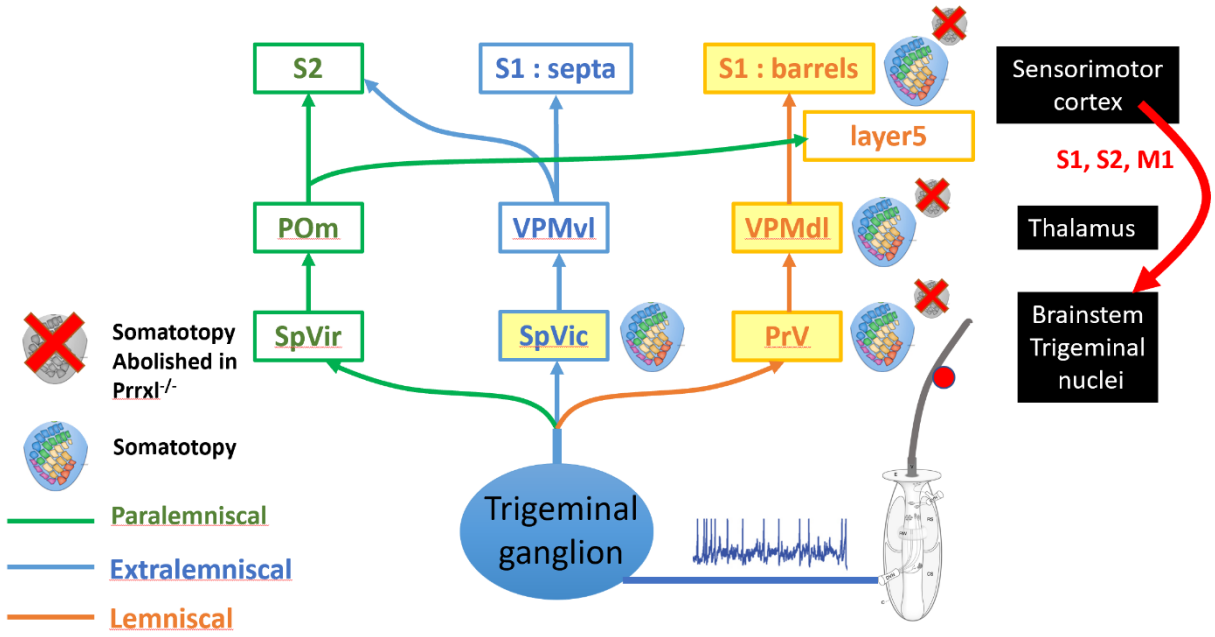


Figure 3.1 Ascending parallel somatosensory pathways.

Feed-forward simplified pathways of the vibrissae-barrel neuraxis. 3 labeled pathways are shown, colored separately. 2 of the 3 ascending pathways, lemniscal and extralemniscal display topography as evidenced by somatotopic arrangement in respectively labeled nuclei. In the Prrx1$^{-/-}$ mouse model, somatotopy is abolished throughout the lemniscal pathway. Heavy top-down innervation of all pathways exists from sensorimotor cortex into brainstem trigeminal sensory complex (red arrow). Whisker follicle and example spike train upon touch is illustrated as the start of the afferent signal.

3.2 Methods

All methods were approved in advance by the Institutional Animal Care and Use Committee (IACUC) of Northwestern University.

3.2.1 Animals

Subjects were adult wild-type (WT) and mutant Prrxl1$^{-/-}$ (KO) mice based on the CD1 strain. Both female and male mice were used (WT 3 female, 5 male; KO 5 female, 3 male). Range of ages, in months, for both groups was similar (WT 2.3-7.6 starting, 13.6-22.6 ending; KO 4.6-7.6 starting, 12.9-21.1 ending). The Prrxl1$^{-/-}$ mice, also known as the DRG11 line in the literature (Ding et al., 2003; Jacquin et al., 2008), were generated in the Feinstein lab at Hunter College (Bakalar et al., 2015).

Eight WT and eight KO mice were transferred to Northwestern University from Hunter College to participate in experiments on both drinking and feeding behaviors. Mice were at least nine weeks old at the time of transfer to Northwestern and 3-20 months old during data collection. Transferred animals were housed in a reverse light cycle room, 10hr dark:14hr light, with their dark cycle starting after 8am CST. Mice from same litter were co-housed (paired) in a cage for social enrichment.

For details on the Prrxl1 knockout line, see Chapter 2 methods.

3.2.2 Experimental setup

The behavioral paradigms were designed to encourage mice to search in a volumetric space and interact with objects placed in that space. The experimental setup, depicted in Fig 3.2, consisted of: 1) a rectangular transparent acrylic elevated (2ft) enclosure (8”L×2”W×4”H) with single entrance/exit point; 2)

2 high-speed 300 frames per second monochromatic cameras (Dalsa Genie HM640) fitted with zoom lenses; 3) an array of custom built high-power infrared LEDs (IR, >750 nm) placed in flexible gooseneck mounts which provided backlit illumination; 4) a robotic arm controllable in real time by the experimenters (Dobot Magician, DOBOT); 5) A series of interactive objects including custom built acrylic platforms, hypodermic tube poles, or 3D printed shapes mounted to the robotic arm end effector; 6) custom built beam break circuits that triggered entry/exit of an animal at the edge of the enclosure and 7) a microcontroller (Arduino Mega2560) that ran the paradigm's state machine, triggered cameras, detected beam breaks and powered the IR sources.

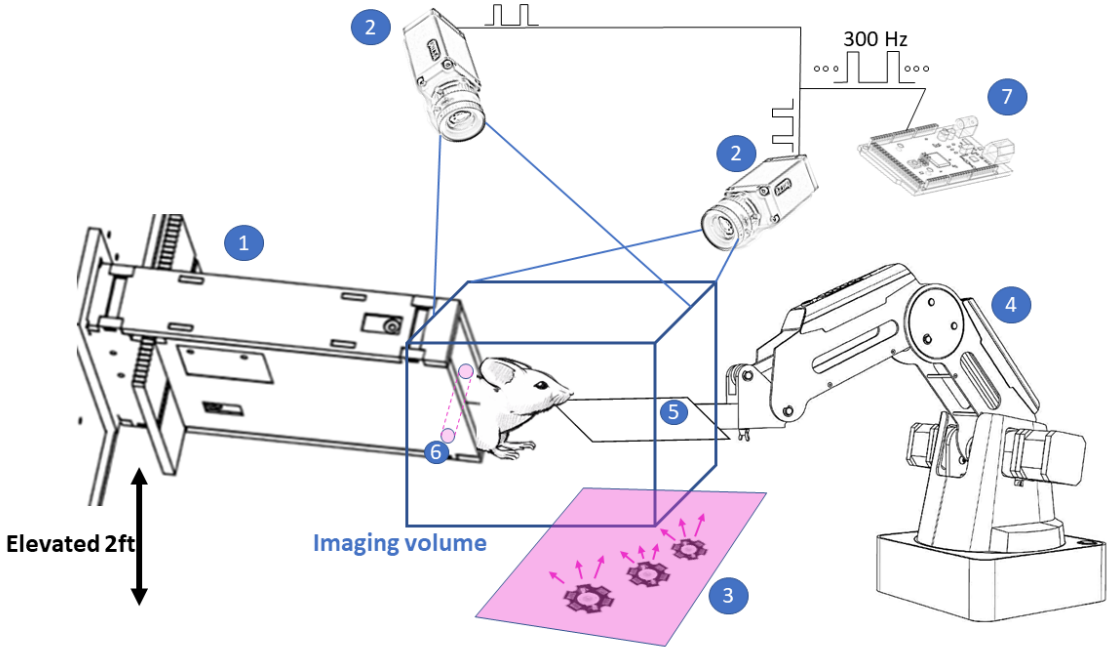


Figure 3.2 Experimental setup. Components and layout.

1. Enclosure with one open entry/exit; 2. 300 fps cameras; 3. custom-built IR (>750nm) array for backlit illumination; 4) a robotic arm placing interactive components in the volumetric space; 5. example custom-built jumping platform placed on the robotic arm; 6. Beam-break circuits that triggered entry/exit of an animal; 7. master Microcontroller running the behavioral paradigms state-machine.

The acrylic enclosure was immobilized onto an elevated platform of about 2 feet from the surface of a tabletop. It provided a housing where the mice could choose to retreat for safety or breaks. Each trial was

voluntarily initiated by the mouse by extending their head out of the enclosure. No explicit cues were provided, but movement of the robotic arm could have constituted a cue and initiated an exploratory bout. When the mouse head extended beyond the enclosure entrance, a beam break was triggered and a video capturing TTL trigger relayed to the high-speed cameras.

The cameras were placed so that they provided a top-down and left-view of the volume of space where the mouse could perch and explore in contact-free air or interact with proximal environment features such as an edge of a platform or a vertical pole (Fig 3.3). Video acquisition was synchronized via TTL pulses sent simultaneously to both cameras by the microcontroller and conditioned on the triggering of the beam break positioned at the entrance of the enclosure.

Manual starting of video acquisition was initiated by the experimenters for trials where the mice were on the platform, outside the enclosure, and jumping back into the enclosure. Manual confirmation of synchronous capture was done prior to experiments initiation by triggering an LED and observing the simultaneous capturing of its onset and offset on both views.

Figure 3.3 Stereo vision capturing of volumetric haptic behavior.

Both left view and top-down camera views are shown. A vertical pole stimulus is also present in the frame, controlled by the robotic arm. Schematic shown as inset on the left panel. Whiskers are visible in both views. The mouse body is perched, extended forward, front paws grasping the edge of the enclosure, and the head is pitched down.

Video data was acquired using a commercial frame grabber and recording system (Streampix, NorPix). The software was setup to acquire any incoming frames and since frames were triggered externally by the Arduino microcontroller, no communication was needed between the Arduino and Streampix software. Instead, the experimenters enabled recording manually for each trial by using the record function of Streampix, following which any triggered frame was simply recorded on file. Experimenters checked that number of frames matched between the two views during recording sessions. Each trial generated a video file consisting of an epoch of time starting with the mouse extending their head out of the enclosure and ending when they retreated voluntarily back into the enclosure. These trials typically lasted from a few to dozens of seconds.

The captured volumetric space was backlit illuminated using an array of 3 high-powered IR LEDs for each view, custom assembled with heatsinks, goosenecks for easy placement and optimization of illuminating space, and powered with a current driver (BuckPuck 3023-D-N-1000, Luxdrive) gated by the Arduino microcontroller. Diffusion filters (BH photography) were placed in front of the high-power IR LEDs, with several grain and pattern sizes stacked to manually produce as much of a uniform backlit background as possible. Since the mice had a white coat and translucent white whiskers, backlighting was necessary to obtain enough contrast. This also constrained the number of cameras that could be used, as only two orthogonal illumination planes could be created that did not interfere with each other.

Experimenters were located outside the behavioral test room and remotely controlled the robotic arm and acquisition of the cameras. They monitored the mouse behavior through a low frame rate, 30fps, night vision camera. The experimental room was fully darkened by using black tape and fabric and manually confirmed by the experimenters dark adapting in the room for about 15 minutes and confirming no light leaks or sources.

3.2.3 Behavioral paradigms

At the start of the experiment a mouse was placed inside the acrylic enclosure either manually or placed on the robotic arm platform and encouraged to perform a gap crossing and jumping into the enclosure themselves by placing the platform near the enclosure. The enclosure provided a means for the mice to

retreat to a ‘safe’ space. As a result, all mice resolved to a stereotypical behavior where they would perch out of the enclosure, explore the space by whisking, sniffing and head movements in all three dimensions and quickly retreat to the safety of the enclosure.

All the paradigms described below made use of this stereotypic behavior. That is, all data captured naturalistic haptic volumetric exploratory behavior, that was neither cued nor instrumentally trained. As all trials were self-initiated by the animals, the number of trials per animal varied across days and across animals. We limited sessions to a maximum duration of one hour and ended them early if the mice were not willing to explore in a period of 10-15 minutes.

During each session a mouse performed one of the following described paradigms, which was defined by the haptic environment created by the end effector placed on the robotic arm.

3.2.3.1 Contact-free air whisking

For this behavior paradigm the trials consisted of epochs of time when the mice perched out of the enclosure and whisked in free air. No contacts occurred with environment features. These trials were randomly intermingled between the other paradigms described below and designed to capture the kinematics of head and whisking in contact-free settings. Mice were typically motivated to perch out and whisk in space as they expected to detect and explore environmental features. During these trials the robotic arm was retracted, and its end effector positioned at least 2 feet away from the mice and at the edge or out of the field of view of both cameras.

3.2.3.2 Vertical pole localization

A vertical hypodermic cylindrical miniature tube (Fig 3.3) was affixed to the end effector of the robotic arm or below and extending beyond the edge of an acrylic horizontal platform, also affixed to the end effector. The tube’s height was up to 5cm long.

As mice-self initiated an exploratory trial, they were free to contact the pole and either whisk against it or if proximal enough try to grab it with their paws. The location of the pole was manually shuffled by the

experimenter every few trials to cover both sides and a range of heights in the volume of haptic exploration space.

3.2.3.3 Horizontal pole or horizontal edge localization

Horizontal features in the environment were provided for mice to localize, orient, or haptically interact. Either a pole or a horizontal platform edge was positioned in front of the enclosure. The pole (same dimensions as in 3.2.3.2) was affixed to the end effector or the acrylic platform and oriented horizontally. The length of the protruding pole was up to 10 cm.

As mice-self initiated an exploratory trial, they could contact the pole with either their whiskers or if proximal enough try to grab it with their paws. The location of the pole was manually shuffled by the experimenter every few trials to cover both sides and a range of heights.

During trials that included the platform, mice were free to jump on it, performing a gap crossing following initial localization. When the pole was affixed to the horizontal platform, it was sometimes used as a lever to aid in gap crossing.

3.2.3.4 Platform jumping and gap crossing

A rectangular acrylic platform (Fig 3.2, feature #5) was affixed to the end effector of the robotic arm and positioned so that it created a gap from the entrance of the enclosure. The gap size and height offset from the enclosure floor was changed between trials and the platform placed at different positions in order to increase spatial arrangement variability and decrease predictability for position, orientation and gap size. Mice perched from the enclosure, and upon localization of the platform edge with their whiskers they were free to extend their body and perform a gap crossing or platform jump.

Subsequently, this behavior was mirrored on the return trip. Because the platform was affixed to the robotic arm and positioned in an opened air space, mice only spent a few seconds on it and immediately started to try to get back to the safety of the enclosure from which they just jumped onto the platform. The animal, now on the platform, needed to orient itself in the other direction and detect walls and/or floor of the enclosure and perform a jump back into its space. Varying the height of the platform between trials,

encouraged the animals to pitch their heads up and down and sense environmental features such as enclosure walls, aperture and location of the horizontal floor surface. All platform jump trials, included a jump back into the enclosure trial, effectively doubling the trials and behavior.

3.2.3.5 Aperture detection and vertical space localization

A portion of the platform jumping trials (3.2.3.4) were effectively reconfigured as aperture detection and vertical space localization trials. In these trials, the aperture space back into the enclosure was blocked by the vertical pole affixed to the platform. When the mouse jumped to the platform from the enclosure, the experimenter moved forward the platform via the robotic arm and used the vertical pole affixed to its end to block the aperture of the enclosure. This divided the space of the entrance into a smaller and larger opening. Mice were able to sense this division after whisking against it, adjust their head orientation, and extend their bodies towards the openings which afforded entrance back into the enclosure.

All trials that included a platform jump, also included a jump back into the enclosure and it is on this back jumping trials that the aperture detection behavior was performed.

3.2.3.6 Shape habituation

A custom-made four-pronged holder was used to place 3D printed shapes on its prongs. The setup was affixed to the end effector of the robotic arm and positioned such that animals could not readily jump onto it and had to extend significantly out of the enclosure to whisk against the shapes.

Each shape was either a hemisphere or a rectangular prism with a square face of the same dimension as the diameter of the hemisphere. One provided a gradient of depth and smooth surface, the other provided a flat surface and sharp edges to whisk against.

Each trial consisted of either the same shape replicated on all four positions, or three of the shapes same and one of the shapes different. This shape habituation task required the mouse to perch from the enclosure and whisk against any or all the shapes. No rewards or cues were provided, and animals were free to self-initiate each exploratory trial.

3.2.3.7 Localization and orienting towards a water reward

The data from these trials comes from the licking experiments descripted in Chapter 2. These trials are similar to horizontal pole detection trials 3.2.3.3, but they include a reward. As described in methods in Chapter 2, animals were water deprived and maintained at a regimen of 1ml/diem.

Each trial consisted of a mouse perching out of the enclosure and detecting and subsequently orienting its head towards the licking spout. Upon spout detection, animals performed a series of licking events. Immediately after the first lick was detected one drop of water was delivered. Animals could stay near the licking spout, wait an intertrial interval of at least 5s and start a second rewarded trial, or they were free to withdraw into the enclosure and after some time start another exploration and water consumption trial.

In this chapter we focus on the localization and orienting aspect of this behavior, not on the licking and water consumption aspect. This paradigm differs from all the other paradigms as it includes an explicit goal and reward. Localizations and orienting trials are in the context of a reward seeking, goal-oriented behavior.

3.2.4 Head and whisker kinematics processing

We have been developing tracking code to tackle this large collection of naturalistic behaviors. A recent artificial neural-net based tracking tool, DeepLabCut (Mathis et al. 2018), is being used to automate the process of extracting whisking parameters on a frame-by-frame base. The workflow involves manual labeling of a small set of frames (~200) for features of relevance and training the neural net to generalize the image segmentation and labeling process in other frames of the same camera view.

We have focused on a small subset of facial features (eyes, nose, and midpoint between eyes) from which we plan to compute head orientation and velocity parameters. Rostral and caudal whiskers are also labeled in a separately trained network, from which we compute the average angle and spread of each array (Fig 3.4). The average angle of each whisker array is independently tracked and the whisking signal temporally smoothed, with filter parameters being optimized.

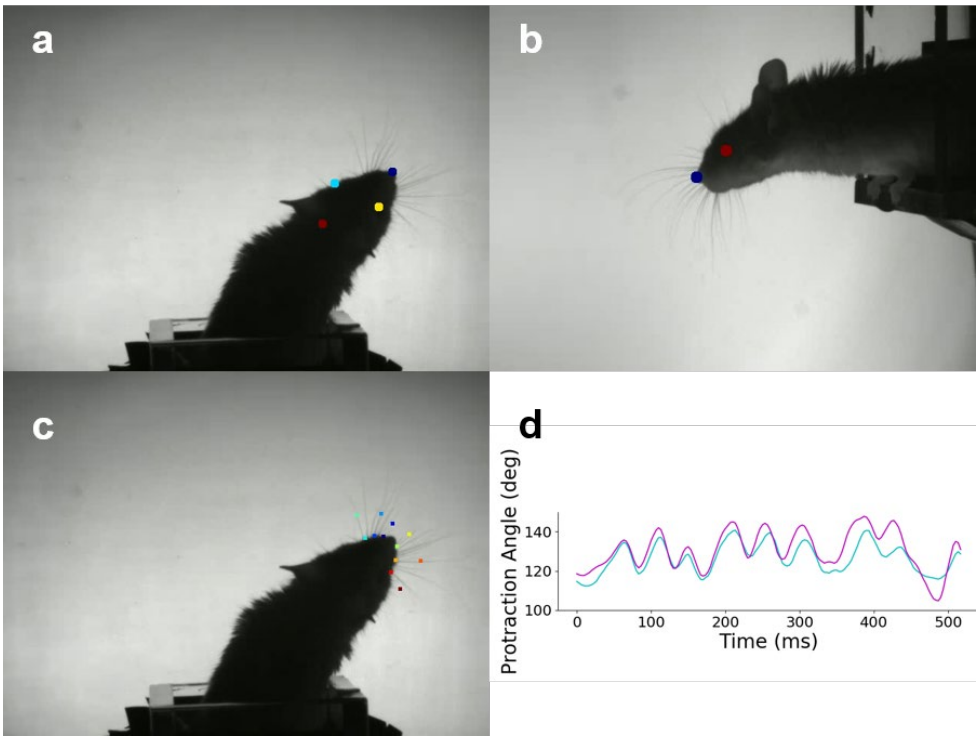


Figure 3.4 Automatic tracking of facial features and whisking kinematics.

Labeling of facial (**a, b**) and whisker features **(c)** is shown, with each colored dot an individually segmented feature with DeepLabCut**. d)** Extracted whisking time course during 500ms of whisking. The two different colored lines represent the mean whisking position in degrees relative to the animal's sagittal midline, computed separately for the two whisker arrays. This is an example of synchronous contact-free whisking. Whisking signal computed only from the top-down view.

3.3 Preliminary results from tracking methodology

We have collected video data in 8 Prrxl1$^{-/-}$ and 8 WT mice. The immediate goal is to use the distance between either the nose or estimated whisker array volume (Huet & Hartmann, 2014) and a haptic object of interest. Head velocity profiles are planned to be used in inferring contact epochs, with head velocity expected to display sharp changes upon haptic interactions, as shown in rat freely moving studies (Arkley et al., 2014; Carvell & Simons, 1990; Mitchinson et al., 2007; Von Heimendahl et al., 2007). Orienting and localization behavioral performance in all the paradigms tested, will compare wildtype and mutant

animals. This includes success rates in jumping platform paradigms, successful head turns in the vicinity of a haptic feature, and distance from head to the jumping platform.

By using stereovision and performing a camera calibration step with the aid of a checkerboard, information from two views can be 3D merged for each frame in which it is possible to identify the same feature in both camera views (Fig 3.5). We plan to use interpolation for frames where features are not detected, within some behaviorally relevant window (e.g. 10ms for whisking, longer for head movements). Extracting information from two views reduces error from depth of field uncertainty that would be present if only one view was used. It also allows quantification of head kinematics in 3D and more accurate contact location and time estimations. In addition, this dataset allows to quantify whisking behavior as a function of head pitch, which no study known to me has previously studied.

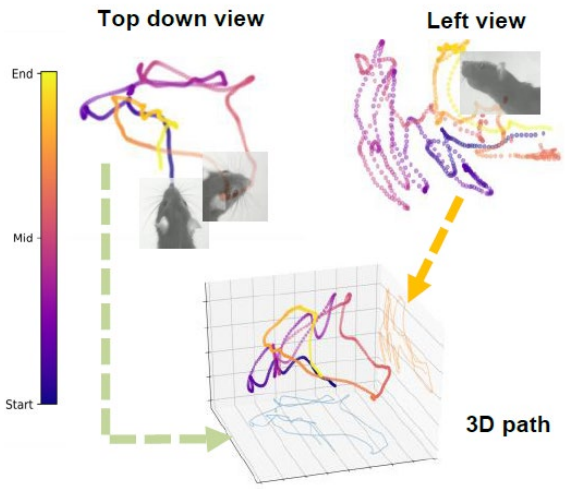


Figure 3.5 Reconstruction of head kinematics in 3D reveals complex turns in volumetric space.

The path of an animal's head perching out an elevated enclosure is estimated from the tip of the nose automatically extracted from video frames. Two 2D views are compared in the bottom panel with the full 3D path after stereo-vision merging. Considerable pitching of the head is observed from the left view, with the 3D path showing head orientations embedded in 3D space and complex turns that include both yaw and pitch. Data colored by progression through the trial.

Preliminary analysis comparing Prrxl1$^{-/-}$ and WT mice whisking from top-down videos in contact-free air indicates that whisking kinematics in the mutant remain largely intact (Fig 3.6). The distribution of both genotypes show the same spread and peak at the same frequency. This small sample of data was computed from two individual mice (one from each group) so no statistics are shown. The wild type animal happened to do a longer session and more whisks are analyzed. Extending this result to a small

sample from multiple mice is the next step. This preliminary data, if held true when multiple animals are analyzed, suggests that deletion of the Prrxl1 gene and disorganization of the leminscal ascending sensory stream does not impact the primary features of whisking behavior in contact-less free air. We also quantified the amplitude distributions for this dataset. However, due to uncertainty of estimating amplitudes when animals are pitching the head, we cannot yet say that the distributions are the same.

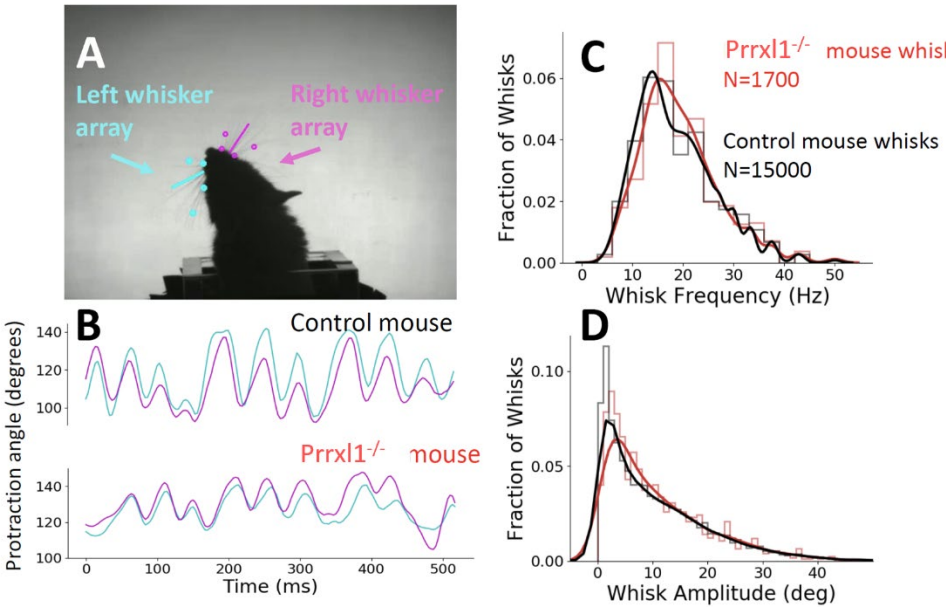


Figure 3.6 Contact-free whisking kinematics appear to be largely intact in Prrxl1-/- mice.

A. Individual array midpoint location is computed for each array (solid line, relative to sagittal midline axis) and shown in separate color. **B**. A sample time course of whisking signal for a WT(top) and Prrxl1$^{-/-}$ animal (bottom) shows no qualitative differences. **C-D** Both frequency and amplitude distributions are similar between mutant and control animal. The WT animal had a longer session with more whisking bouts occurring.

3.4 Discussion

3.4.1 Untethering behavior: The need for naturalistic freely moving experiments in mice

In order to better model the motor system and action space, we need to capture natural behaviors in sufficient detail and over sufficient variability which encompasses the underlying distributions of kinematic variables employed by the organism when not perturbed and not restricted. Overcoming this challenge is especially paramount for the rapid and dynamic active sensing rodent whisking behaviors, which occur in concert with body and head movements.

Head-fixed rat behavioral experiments have uncovered not only the ability of animals to modulate key whisking parameters, but also use of fundamentally different strategies that depend on task parameters. Simple object detection and learning a detection task correlates with number of whisks emitted, whereas harder tasks of shape and texture discrimination display a more focused strategy, with animals increasing frequency of whisking and palpating at lower amplitudes repeatedly against the object (Carvell & Simons, 1995; Harvey et al., 2001). Poor task performers differ in the temporal whisking patterns from good performers (Carvell & Simons, 1995). Classical conditioning studies show ability of head-fixed rats to learn to vary basic kinematic frequency and amplitude parameters of whisking in a stimulus-dependent manner (Bermejo et al., 1996; Gao et al., 2003), strongly suggesting voluntary control of this active sensing behavior.

Task and context-dependency of frequency, angular spread, and the resting/starting protraction angle of whiskers in rats (Carvell & Simons, 1990; Grant et al., 2009; Sachdev et al., 2003; Sellien et al., 2005) show that adaptations of the sensory array itself while engaged in the behavior, if sufficiently characterized, can be used to answer questions of whether animals are using them to optimize object contact and localization. Such characterization and modeling would provide support for the evolutionary adaptation of whisking muscles and movement repertoires.

This modulation of whisking parameters is also shown in freely moving rats. Switching modes from exploratory whisking with large amplitudes and slower frequency to more foveal whisking of small amplitude at protracted angles and larger frequencies is matched by differential muscle activations recorded by EMG (Berg & Kleinfeld, 2003). The loss of sensory feedback via nerve transaction had no effect on the muscle phasic activations over the full frequency range, suggesting a top-down control and ruling out a simple scheme of multiple reflex arcs.

In addition, neural correlates of complex haptic environments with texture, in primary somatosensory cortex are diverse (Von Heimendahl et al., 2007), with a preference for stimuli with higher statistical features (rougher vs. smoother texture). This correlates with response choice of the animal, switching to firing rates higher for smoother stimuli in incorrect trials. Yet, there is ambiguity in single and population neural responses, with units firing for both conditions and in periods when not in contact, suggesting other sources of input, like cortico-cortical feedback signals or signals related to motion of whiskers or head. Prior anesthetized studies have shown bilateral input at the stage of primary somatosensory cortex, with neurons in layer 5 modulated by ipsilateral stimulation, with this influence being subthreshold and disappearing after inactivation of contralateral S1 (Shuler et al., 2001). This input could be influenced by well-known M1 projections to S1, coordinating bilateral whisker sampling.

Different sampling strategies are employed under different behavioral conditions on trained discrimination experiments. On a gap crossing and texture discrimination task, blindfolded rats move their whiskers to discriminate and lower amplitudes and increase contact with a textured surface, with some whiskers continuously bent in the process (Carvell & Simons, 1990). When a gap is placed vertically creating an aperture, placement of the head and array is sufficient to discriminate with the whiskers but does not require movement of whiskers themselves as confirmed with facial nerve lesioning. Trimming all the whiskers drops discriminability to chance, and this effect is graded with the performance depended on number of intact whiskers (Krupa et al., 2001), demonstrating the necessity of multiple whiskers for at least some laboratory tasks and suggesting other cues in play like head positioning or orientation.

Freely moving rats show, in addition to whisker movements, multimodal sampling strategies with rapid increases in sniffing rate, periods of significant head movements and periods of head rest, in addition to active movements of the nose (Welker, 1964). Modification of the haptic environment and its affective association elicits modification of exploratory behavior. One trial with 3 mild foot shocks is sufficient to condition a rat to avoid arms of a 'Y' maze that contain vertically oriented wall slats as the conditioned stimulus, with conditioned response disappearing when changing to no difference between conditioned and unconditioned stimuli, and whiskers required for the conditioned response (Polley et al., 2005). Behavioral strategy changes, within a task, is shown in blind rats running down a maze to obtain reward. Modification of environment context to include unexpected obstacles result in rats changing sampling strategy, with reduction of running speeds protraction of whiskers forward while running in 'look-ahead' fashion (Arkley et al., 2014). A change in head pitch (lift) is observed in sighted rats as animals learn the same task, with animals maintaining a higher pitch head orientation as they start to run faster with an increase in task familiarity.

Coordination of whisking with head movements is shown in asymmetric modulation of whisking in exploratory and goal-oriented behaviors (Towal & Hartmann, 2006). This asymmetric control of whisking can happen during object detection and in the same whisk cycle as the initial contact (Mitchinson et al., 2007). Whisker protraction ipsilateral to the side of contact with an object is depressed, while the contralateral side tends to protract at higher amplitude. These studies suggest that whisker and head movements are actively controlled to increase the likelihood of environmental contacts (Grant et al., 2009).

The ethology of whisker and body/head coordination has been shown in different species. The Etruscan shrew localizes and captures quickly moving crickets with the use of its whiskers and head movements, and shape of the cricket alone is sufficient in driving predatory attacks, whereas vision and olfaction do not elicit a behavior (Anjum et al., 2006). Similarly, rats do not necessitate vision for predatory behaviors that require localization, identification, and coordination with the oral motor programs (Gregoire & Smith, 1975).

All the above findings in freely moving rats have yet to be replicated in the mouse model, currently the most advanced in terms of genetic access, large scale optical and electrophysiological recordings, and opto- and chemo-genetic perturbations of neural circuits. Recent head-fixed mouse experiments have shown surface, shape, localization and object orientation discriminations (Brown et al., 2021; Hires et al., 2015; Kim et al., 2020; Rodgers et al., 2021).

Naturalistic experiments that allow the animal to use its own sampling strategies and which do not restrict degrees of movement freedom would further inform how to model the behavior so that underlying computations can be hypothesized and tested against the neural data. This is especially important for the dynamic active sensing whisking behaviors, where an animal can scan locations and decide which contains a stimulus within a whisking cycle (Mehta et al., 2007).

3.4.2 Tracking whiskers: advances in methods and feasibility for full array experiments

Capturing the details of whisker movements has been a challenge that still has no good generalizable solutions for freely moving, naturalistic behaviors. Whisker arrays can move at high velocities in cycles of 5-15Hz in contact-free air and adapt to smaller amplitudes with significantly higher frequencies (15-25Hz) upon contacting an object of interest (Berg & Kleinfeld, 2003; Carvell & Simons, 1995; Harvey et al., 2001). Textures and frictions can generate slips on the whisker tips (Arabzadeh et al., 2005; Neimark et al., 2003; Ritt et al., 2008; Wolfe et al., 2008), with high-frequency vibration events present during natural whisking even in contact-free air (Wolfe et al., 2008).

There is no good single angle to view and disambiguate individual whiskers in a whisker pad as rows and columns overlap each other in a video frame. A high-speed camera placed on a fixed orientation and capturing a specific projection of the rodent head and whiskers, immediately runs into ambiguous configurations of the 35 individual whiskers on each mystical pad. This problem is made harder in a freely moving setup, as the animal rotates its head on all axes - pitch, yaw and roll. As an example, if a top-down camera view observes a mouse head oriented in the horizontal plane, columns of whiskers are observed and rows of whiskers are occluded, with individual whiskers in a column aligned and partially

occluding each other. When the animal pitches their head down, all columns of whiskers start to overlap each other, with the new projection of whiskers on the camera now displaying a separation by rows. As the animal actively whisks, more complexities are added. Rotation or resizing of the whisker pad has been reported (Haidarliu et al., 2010; Knutsen et al., 2008; Towal et al., 2011).

Any attempt to accurately model the movement of the array or individual whiskers, in freely moving animals, requires a well-estimated orientation of the head. Only then can models of whisking (Knutsen et al., 2008; Zweifel et al., 2021) and whisker arrays (Belli et al., 2018; Belli et al., 2017) be superimposed on the rodent's head and provide the crucial kinematic parameters of angles (amplitude), velocities, and ideally spread of the array, from which further higher-order features such as whisking frequencies can be accurately estimated. To obtain the orientation of the head in a freely moving naturalistic experiment, at least two synchronized cameras are needed.

It is then not surprising that restrained preparations are used to further the capabilities of tracking whisking behavior, with the aim of real-time feedback experiments made possible. Single whisker trajectory tracking has been possible with opto-electronic methods that are very fast and can be used in theory for online real-time processing (Bermejo et al., 1998; Chakrabarti & Schwarz, 2018; Hentschke et al., 2006; Wolfe et al., 2008). However, it is the flexibility, accuracy and full whisker description made possible with high-speed video capturing that can enable full characterization kinematics along the whisker length and ultimately estimate the 3D mechanics at the whisker base (Hires et al., 2016; Huet & Hartmann, 2016; Knutsen et al., 2008; Yang & Hartmann, 2016). Knowledge of whisker shape and its kinematic variables is a necessary input for all models that estimate moments and forces that mechanosensory receptors ought to be experiencing. With ability of mice to move whiskers individually (Dörfl, 1985; Mehta et al., 2007; Simony et al., 2010), tracking of individual whiskers is desirable.

Multiple algorithms exist for single whisker tracking from high-speed, high-fidelity videos. Image processing and machine vision can be used to identify and link locally linearized line segments representing individual whiskers, with tools that require minimal supervision such as the Whisk program (Clack et al., 2012) available. These tools work well in a fixed frame head-fixed setup and have been

used by multiple laboratories, including ours. A higher signal-to-noise image processing pipeline algorithm, WhiskEras, has improved on Whisk (Betting et al., 2020) and aims to extract individual whiskers from untrimmed whisker arrays. While promising, this pipeline has yet to be tested in other laboratories and is under active development. Most importantly, it is optimized for head-fixed, contact-free whisking with a static background that currently does not allow objects and movements of objects in the field of view.

A separate impressive effort that makes use of fitting 3D Bezier curves to the basal section of each target whisker has recently demonstrated tracking of multiple whiskers in a head-fixed setup and capturing full three-dimensional kinematic variables, including whisker 3D shape and orientation (Petersen et al., 2020). This setup involves stereo-vision capture of two views via two high-speed cameras, a necessity for any three-dimensional characterization of whisker kinematics. Alternatively, careful geometric positioning of mirrors that project secondary image planes, followed by finding corresponding points in both views can be used to extract three-dimensional shape (Kim et al., 2020).

While awake, behaving, head-fixed paradigms make it feasible to track whisker kinematics in fine detail, at least for a row or a few whiskers, these experiments miss the important vestibular contribution of the head movements and restrict the animal's own degrees of movement freedom. The restrictions imposed by head-fixation invariably modify the sampling strategies that animals can use compared to the unrestrained, freely moving condition. Moreover, horizontal plane characterization, ubiquitous in head-fixed experiments, only measures one of the 3 angles that define whisker array orientation, while it is well known that whisker movement contains a roll component about its longitudinal axis creating significant torsional components (Knutsen et al., 2008; Knutsen et al., 2005). The same problem holds true for head movements captured by one camera in top-down videography in freely moving setups. Roll and pitch of the head modifies the apparent whisker angles recorded by video capture, and head turns have been shown to modify whisking behavior itself (Mitchinson et al., 2007; Towal & Hartmann, 2006). Only by describing the behavior in terms of all the kinematic variables can shed light on these relationships.

Mice whisk considerably faster than rats (20Hz in mice vs. 8.4Hz in rats), but at similar mean amplitudes for arrays in contact-free whisking. The fast-whisking behavior of these rodents is due to type 2B fibers, which provide high maximum contraction velocity and are less fatigue resistant (Jin et al., 2004). This fast motor output necessitates high-temporal resolution for capturing of the behavior. At least 100 frames per second (fps) are needed for mice whisking amplitude extraction, with sampling at 50fps severely dampening estimation of amplitudes and phase errors smaller than 5 degrees only possible at >250fps (Perkon et al., 2011).

Unsupervised tracking of both head and full whisker array in freely moving animals has been attempted and image processing pipeline and algorithms described and published as the Vibrissae and Snout Analyzer (VISA) in (Perkon et al., 2011). A GUI tool that makes use of it has been built (BIOTACT Whisker Tracking Tool (BWTT)). This is the only study to date known to attempt image segmentation of an intact full array, together with head kinematics descriptors. This effort unfortunately has been largely abandoned and not taken up by many laboratories. On our hands, the algorithm was optimized only for top-down videos, needed frequent user input to recalibrate parameters, and its whisker segmentation output relies on a Hough transform approach which approximates a user-defined basal portion of a whisker as a shaft and ignores natural whisker curvature. Nevertheless, it is an example that shows the feasibility of tracking intact arrays and head motions of an animal. This can be the first in a series of processing steps that combine with other techniques which better characterize a whisker from multiple camera angles. Optimizations for image processing, parallelization of computations and GPU enabled workflows can push the algorithms towards real-time tracking as demonstrated for the VISA algorithm (Ma et al., 2017).

Other unsupervised methods of tracking multiple whiskers in freely moving rodents exist. Anisotropy tracing of full whisker shapes has been demonstrated, with some invariance to image resolution, sampling rate and contrast. This is one of the most promising methods and used to track individual whisker kinematics and automatic contact detection in a gap crossing task (Voigts et al., 2008). However, only a few whiskers were trackable at a time as this vector fields approach cannot be easily extrapolated

to occlusions and overlap of whiskers. Methods that make use of temporal information, like hidden Markov models and spline fitting have been successfully used to interpolate between frames where information is missing (Clack et al., 2012; Voigts et al., 2008).

For the dataset described in this chapter, it is decided to employ an artifical deep neural network-based approach, DeepLabCut, described in (Mathis et al., 2018). This solution, while not generalizable, has a practical workflow that makes it possible to track both body parts and whisker features. Only about 100-200 manually labeled featured are needed to train a network at acceptable feature identification performance. One caveat of these artificial neural based solutions is that they require training for each view and each object/feature set separately. In addition, it is not clear what image spatial features the network trains on as it iterates over training steps. Validation requires a user to randomly sift through evaluated frames and videos.

Nevertheless, this approach is rapidly progressing, pushed forward by large image datasets and more advance network architectures that promise automatic image segmentation and accurate kinematics estimation of moving features. Combined with multi-camera high-speed behavioral acquisitions and three-dimensional merging of features from multiple views, full array whisker tracking, and head and body kinematics will be possible in the not-too-distant future for the common laboratory.

Chapter 4 : Discussion

4.1 Modeling behavior and capturing kinematics in all three dimensions

Behavior is complex. It occurs over time as sequential movements are daisy chained from activation of muscle synergies. In order to map the functions between an organism's actions and neural space, we need to better model behavior. The behavior investigated in the laboratory it is thus desired to be complex enough and span sufficient range so that the extracted kinematic variables cover the variance of natural movement chains. Some species, like aquatic and flying animals perform volumetric navigation. Even for terrestrial animals, like rodents, body movements are embedded in a three-dimensional (3D) space. As rodent navigate and explore proximal space with their vibrissae, for example while burrowing or climbing, their head onto which the whisker arrays are constrained, rotates in all three principal components: yaw, roll and pitch. Studying the natural complexities of movement and correlating models of it with neural representations can aid in uncovering general principles of sensorimotor loops.

Much of the research in Neuroscience has focused in describing and uncovering transformations from stimulus space to neural space. However, even for the most studied modality, vision, its high dimensional space makes it hard to narrow down higher-order features, like object shape, in neuronal space (Yamane et al., 2008). Even when a neuron's output is characterized as a function of simpler stimulus features, its selectivity or the selectivity of a population of similar neurons to any higher-order features can only be done with abstract modeling (Kouh & Poggio, 2008). The entirety of rodent whisker sensor and action space, receiving input from a small discrete set of facial follicles which are actuated by a small set of muscles, provides a lower dimensional representation for trying to characterize sensorimotor behaviors in terms of neuronal ensembles. Overcoming the challenge of modeling the action space is especially paramount for the rapid and dynamic active sensing rodent whisking behaviors, which occur in concert with body and head movements.

Neural head direction (HD) representation (Raudies et al., 2015; Taube et al., 1990) has been one of the main findings for representations from both idiothetic (self-motion) and allothetic (external landmarks and

movement) cues, and relationship of self with the environment. HD cells fire as a function of the animal's head direction and exist in multiple brain areas (Taube, 2007). This representation complements allocentric information neural correlates; place cells (Ludvig et al., 2004; O'Keefe & Dostrovsky, 1971) code specific environment-context locations; grid cells (Hafting et al., 2005) form a tessellated hexagonal lattice of environment space; and border cells (Solstad et al., 2008) represent environment boundaries such as walls. Interestingly, they seem to not only represent but also predict future head-orientation (Meer et al., 2007), especially unexpected passive rotations induced in the laboratory (Bassett et al., 2005). Brain areas and cell types that code joint distributions of these and other spatial parameters like angular velocity have been reported (Bassett & Taube, 2001; Cacucci et al., 2004; Sargolini et al., 2006).

Head direction system has been described mainly in planar environment experiments and are thought to primarily encode directional heading in two dimensional (2D) horizontal planes, with some modulation by complex rotations in multiple axes (Shinder & Taube, 2019). This view has been challenged by modeling the data for 3D representational models where HD cells integrate information along all three rotational axes (Laurens & Angelaki, 2019). But this is also rebutted (Taube & Shinder, 2020) and there is no current consensus on the 3D representational capacities in rodents for HD cells. Regardless, this does not preclude 3D representations in other cell types or integrating multiple representations, especially given that neurons representing head pitch are reported (Stackman & Taube, 1998). 3D representations in inherently volumetric behaviors, such as flying in bats, are well established (Finkelstein et al., 2015).

Not only the head, but individual facial features move in 3D. This includes the whisker system. Rodent have muscles that allow their noses to rotate about two planes (Deschenes et al., 2015; Kumikova et al., 2017). This allows mice to synchronize three-dimensional kinematic rhythms with sniffing during olfactory search (Findley et al., 2021). The tongue too is controllable on multiple axes (Cortopassi & Muhl, 1990; Kier & Smith, 2008), and licking behavior includes fast and precise corrective submovements of the tongue that are cortex-dependent (Bollu et al., 2021). Similarly, whiskers move in 3D with significant torsion component around main whisker axis (Knutsen et al., 2008; Knutsen et al., 2005) and the whole array can modify it's spread during surface exploration (Grant et al., 2009). Active modification of the

volume of space, for which the array has access (Huet & Hartmann, 2014), can be used as a proxy to study sampling strategies and better model the entire afferent input of a whisking cycle.

Capturing a dataset of 3D freely moving behaviors is only the first step. Extracting the kinematics of whiskers and body parts and characterizing the behavior is the second step that can then feed models of behavior for more rigorous hypotheses testing. Recent advances in automatic image feature segmentation, which make use of very large pre-trained imaging datasets and deep artificial convolutional neural network architectures (Mathis et al., 2018), make the body part feature extraction feasible. These tools have been extended to multiple behaviors, 3D capabilities, and toolboxes of modules are actively being built and made available to the community (Arac et al., 2019). This is the approach we undertook for extracting the kinematics of movement from the volumetric haptic exploratory behaviors we have recorded.

Identification, classification and granularity thresholding of meaningful behavioral descriptors is a challenging problem. Machine vision techniques and data that includes depth descriptors (i.e. 3D) has been used to automatically segment structure in freely moving mouse behaviors (Wiltschko et al., 2015). In these sparse, feature-limited environments, mouse pose dynamics can be predicted from principal modules of behavioral descriptors and their transitions. This autoregressive hidden Markov model workflow can be used in principle for any dataset, including those obtained in more feature-rich volumetric searching behaviors. Behavioral syllables autoextracted can then be curated from expert users in semi-automatic fashion. The set of these syllables were shown to be able to classify phenotypes of different mouse genotypes (Wiltschko et al., 2015).

The true and multi-spatiotemporal scale of behaviors would still be very difficult to extract from such models, which impose bias based on the Bayesian nonparametric approach at specific timescales. The data fed to the models would bias the outcome. That is, how the data is captured (e.g. how many kinematic features) and its quantity would both shift the model results towards either specific behavioral syllables or discovery of larger sets of syllables as data size increases, making cross-strain and cross-species comparisons harder. Imposing some hierarchical modeling and constraints, in iterative fashion,

might resolve some of these issues, and such approaches have been shown to work in large datasets (Allard et al., 2012; Vogelstein et al., 2014).

Even when enough data in freely moving animals and modeling of it is available, if the task acquisition imposes a bias, and the trained task is repetitive, and restricts the movement patterns, the behavioral statistics and function of whisking behaviors concluded from them can be misleading. Brecht et al. (Brecht et al., 1997) was one of the first to show shape recognition in freely moving rats and crucially provided a morphological and likely functional distinction between macro- and microvibrissae. Based on a cookie shape search task, they hypothesized that macrovibrissae in rodents were only used for localization, while microvibrissae were only used for shape discrimination. However, their setup involved a matrix of square cups, with an odd triangle cup, placed down on the ground and whose size afforded easier search by the microvibrissae. They concluded that macrovibrissae were not needed for shape discrimination and the strategy of sampling was serial search, with no head movements needed while sampling. However, all these parameters were already pre-imposed by the task design. By varying tasks and parameters, ideally in non-instrumental paradigms and volumetric environments that do not restrict degrees of movement freedom, we can ask the animals to employ their preferred, optimal strategy. This is the approach we undertook in chapter 3.

4.2 Parallelization of sensory streams: What vs. Where vs. When?

As sensory evidence gradually accumulates, perceptible features start to form and separate. Experiments of perceptual mechanisms, for example accumulation of sensory evidence, were typically done in primates and involved tasks where sensory variables and acquisition time were dictated by the experimenters (Gold & Shadlen, 2007; Romo & Salinas, 2001). However, rodents too can weight sensory evidence (Kepecs et al., 2008), recognize separate visual views as belonging to the same object (Zoccolan et al., 2009), and likely construct generalized rules for solving a task (Murphy et al., 2008). Few natural condition experiments, for example in shrew whisking behavior for prey capture (Anjum et al., 2006), have strongly suggested that whisking sensory variables can be selected to aid specific motor strategies (Munz et al., 2010). Given the parallel pathways of ascending whisker somatosensory streams,

the question then arises of what each pathway conveys and how each stream aids perceptual tasks and behavior in general.

Similar to the separation into distinct dorsal and ventral streams in vision (Goodale & Milner, 1992; Schneider, 1969) and their suggested simplified roles of a respective 'where' and 'what' pathway ('where' dorsal stream providing visual input for action and 'what' ventral stream providing visual input for perception of visual objects), a similar scheme has been suggested for somatosensory functional streams (Diamond et al., 2008).

Rodents seek out tactile information by whisking and upon contact palpating against objects (Carvell & Simons, 1990; Hill et al., 2008; Von Heimendahl et al., 2007). The location and identification of the object is then simultaneously transferred by that interaction. Behaviors that support localization function include platform jumping through a gap (Carvell & Simons, 1990; Hutson & Masterton, 1986; Schiffman et al., 1970), aperture width determination (Krupa et al., 2001), and vertical pole localization for both bilateral (Knutsen et al., 2006; Saraf-Sinik et al., 2015) and unilateral comparisons (Hires et al., 2015; Mehta et al., 2007). Object identification and discriminative behaviors include texture (Carvell & Simons, 1990; Guić-Robles et al., 1989; Prigg et al., 2002; Von Heimendahl et al., 2007), shape (Brecht et al., 1997; Kim et al., 2020; Rodgers et al., 2021), and object orientation discrimination (Brown et al., 2021).

As early as the trigeminal ganglion (Vg), three 'behaviorally' different types of neurons exist: those that are active when whisking, those that are active only when touch occurs (whether phasic or sustained activity), and those that are active for both cases (Bush et al., 2016; Bush et al., 2021; Campagner et al., 2016; Szwed et al., 2006). Touch responsive neurons tile the space of stimulus orientation and firing rate adaptive properties. Models with full 3D mechanical input variables are better predictors of neural activity (Bush et al., 2021).

While Diamond et al. (Diamond et al., 2008) refer to parallel streams beyond primary somatosensory cortex S1, an analogy with the visual streams beyond primary visual cortex V1, there is no current evidence for distinct cortical processing streams of localization and identification functions for touch. Rather, there is evidence for mixing of signals as early as Vg via the whisking/touch responsive neurons.

Recently, identified Merkel receptor cell-associated neurons in Vg were shown to be the ones responsive for coding both touch and phase of whisker protraction in awake, head-fixed, behaving mice (Severson et al., 2017). Re-afferent input together with touch ex-afferent input represented along the same neural stream is a convenient coding scheme for localization and identification of tactile features relative to snout. The more complex higher-order features such as shape and curvature, would require integration across multiple whiskers and receptor types.

A separation of sensory streams can rather happen subcortically. Lemniscal, paralemniscal and extralemniscal pathways have some unique properties. First, there is the cytoarchitectonic differences, with lemniscal pathway displaying topographic arrangement of sensory input into barrel-like structures all the way to S1 (Ma & Woolsey, 1984; Van Der Loos, 1976; Woolsey & Van der Loos, 1970). Extralemniscal also displays barrelettes in brainstem, but paralemniscal pathway does not. Barrelette second order neurons tend to have dendritic trees confined within one barrelette (Veinante & Deschênes, 1999). Second, the receptive fields and neural responses of the pathways differ in the respective brainstem nuclei. In SpVir, which lacks barrelettes, neurons display multi-whisker receptive fields and are organized in row-wise elliptical shapes (Jacquin et al., 1989). Responses in the extralemniscal nucleus are primarily phasic to touch (Yu et al., 2006). In the lemniscal nucleus, PrV, both touch and whisking cells are present and neurons respond to one whisker, and to a weaker extend adjacent whiskers (Moore et al., 2015; Yu et al., 2006). Third, projection patterns differ between the pathways. PrV output neurons come from both barrelette and inter-barrelette populations (Veinante & Deschênes, 1999). While SpVir projection neurons are mainly multi-whisker responsive. Single whisker-responsive SpVic neurons, instead, are GABAergic and project to PrV (Furuta et al., 2008). Taken together, these suggest functional streams, representing some possibly redundant features, and which are hierarchical (SpVic to PrV, but not the other way around). The lemniscal pathway sits at the top of the functional hierarchy.

In S1, every stream has opportunity to intermix and be incorporated in sensorimotor circuits with S2 and M1 signals innervating multiple layers across a barrel column with ample cross-layer connectivity. All the signals available for discrimination are present: what, where or even when. Neurons in the extralemniscal

pathway can provide the phasic touch reference signals for comparison to start of motion, or previous palpating touch during epochs of active sensing. So can whisker/touch neurons.

Goodale and Milner themselves concede that the distinction into functionally separate streams in vision was overemphasized (Milner & Goodale, 2008). Both pathways do in fact connect to each other. But, they stress that their original distinction was aimed at stimulating study of functionally distinct output systems, rather than properties of visual input. That is, they relied on lesion and electrophysiological studies for proposing behaviorally relevant functional roles. Both streams can and should receive both spatial and structural information of objects. Visual object invariant categorization requires knowledge of object relationships and where in space the objects are. Conversely, action towards a visual object requires identification of the object itself. It is what happens with both spatial and structural information and how the information is hierarchically transformed that separates the pathways or rather the networks they subserve.

A similar view can be taken for the somatosensory parallel pathways. Testing functionally distinct output of the lemniscal stream is one of the aims of future analyses using the data collected and presented in Chapter 3. Behavioral categorization of the lemniscal stream, whose topographic arrangement is selectively disrupted in Prrxl1-/- model, will allow to make hypotheses on which behaviors require an intact sensory spatial organization along the lemniscal pathway.

Chapter 5 References

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