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Radial Distance Perception in the Somatosensory System of the Mouse

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"Stehlt, mordet, treibt Unzucht - unsere Lehre ist so stark, daß sie aus der Jauche eurer Sünden schäumend helle Bergwässer macht;"

<div align="right">R.Musil</div>

Contents

1. Acknowledgements

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2. Abstract

Mice sweep their whiskers through space to detect, locate and identify objects. Whiskers are elastic thin rods and all mechanotransduction occurs within the follicle at the base of the whisker. This study addresses the questions whether mice can detect object distance along the whisker (radial distance), which strategies they can and prefer to use when discriminating object positions and set out to identify the mechanical variables at the follicle providing information about radial distance.

Head-fixed mice were trained to judge the distance to a pole presented on one side of their head. Whiskers were monitored and tracked using high-speed videography. Mice reported with licking whether the pole was in a position relatively close to the follicle (the go position reported by licking) or more laterally (no-go position reported by withholding the licking response).

Do mice take advantage of systematic differences in whisker length to judge radial distance? Whiskers were trimmed such that B2 and D2 could contact the pole only in the go (proximal) position, whereas multiple other whiskers could touch the pole in both positions. Mice appeared to adopt a binary strategy: if B2/D2 contacted the pole, it must be a go, otherwise a no-go. B2 and D2 were then cut so that each was too short to contact either stimulus. Performance transiently dropped from >80% close to chance levels. Mice therefore used a "labeled line" strategy to judge pole position under these conditions.

We next chose stimulus positions such that for a single whisker (C2) the azimuth at contact was the same for both go and no-go trials. Under these conditions mice performed radial distance discrimination (>90% at 3 mm offsets). Psychometric curves revealed that mice reliably distinguish radial distance offsets as small as 2 mm.

It has been proposed that mice use the rate-of-change of the moment (RCM) acting on the whisker to measure radial distance (Birdwell et al., 2007). To test this hypothesis, mice were trained to discriminate a single proximal go position from a range of distal no-go positions; mice thus had to categorize stimuli. In a subset of trials, a highly flexible pole was presented in the go position; due to the deflection of the flexible pole this mimicked the RCM of the no-go category. The mice were not reliably "fooled" suggesting that other variables, likely axial forces along the whisker, are cues for radial distance perception.

3. Zusamenfassung

Mäuse nutzen ihre Barthaare um Objekte zu detektieren, lokalisieren und zu identifizieren. Barthaare sind elastische dünne Kegel und die mechanische Reizaufnahme geschieht in den Barthaarfollikeln. In dieser Arbeit wurde den Fragen nachgegangen, ob Mäuse die Position von Objekten entlang der Barthaare (radiale Distanz) wahrnehmen können, welche Strategien sie dazu nutzen können und vorziehen; weiters die mechanischen Variablen zu identifizieren, welche die Information über radiale Distanz im Follikel enthalten. Mäuse, deren Kopf fest verankert war, wurden trainiert, die Entfernung zu einem auf einer Seite ihres Kopfes präsentierten Stabes abzuschätzen. Die Barthaare wurden dabei mittels Hochgeschwindigkeits-videotechnik verfolgt. Die Mäuse teilten durch Schlecken mit, ob der Stab relativ nahe am Follikel (die go Position, angezeigt durch Schlecken) oder weiter lateral positioniert war (die no-go Position, nicht Schlecken).

Nutzen Mäuse die inhärenten Längenunterschiede ihrer Barthaare um radiale Distanz abzuschätzen? Die Barthaare wurden auf eine Weise geschnitten, dass B2 und D2 den Stab nur in der go Position (proximal), mehrere andere Barthaare hingegen den Stab in beiden Positionen berühren konnten. Die Mäuse setzten eine binäre Strategie ein: wenn B2/D2 den Stab berührten, so musste es sich um einen go Stimulus handeln, wenn nicht, um einen no-go Stimulus. B2 und D2 wurden daraufhin abgeschnitten und konnten den Stab nicht mehr berühren, woraufhin die Leistung von >80% auf Zufallswerte fiel. Somit nutzen Mäuse eine "labeled line"-Strategie um die Position eines Objekts unter diesen Umständen zu ermitteln.

Als nächstes wurde die Position des Objekts so definiert, dass ein einzelnes Barthaar (C2) bei Kontakt mit go und no-go Position den gleichen Winkel hatte. Unter diesen Umständen konnten Mäuse radiale Distanzen unterscheiden (>90% bei 3 mm Abstand). Psychometrische Kurven zeigten, dass Mäuse verlässlich radiale Distanzen mit Abständen von bis zu 2 mm unterscheiden können.

Es wurde vorgeschlagen, dass Mäuse das Moment des Barthaares zur Radialdistanz-bestimmung nutzen . Um diese Hypothese zu testen, wurden Mäuse trainiert, eine go von einer no-go Positionsspanne zu unterscheiden. Dazu wurden einige Male pro Experiment ein sehr flexibler Stab in der go Position präsentiert. Durch die Flexibilität wurde trotz der go Position das Moment der no-go Kategorie imitiert. Die Mäuse wurden nicht regelmäßig getäuscht, was andere Variablen, möglicherweise Axialkräfte entlang des Barthaares, als Informationsträger wahrscheinlich macht.

4. Introduction

4.1. The organization of the Whisker field

Rodents sweep their large mystacial vibrissae (whiskers) through space to locate and identify objects.

As the whisker itself consists of inert material, sensory perception occurs at the base of the whisker, in the follicle. The whisker follicle anchors the whisker and muscles give rise to whisker motion by moving the follicle. Each whisker follicle is innervated by around 200 sensory nerve cells which have their nerve endings with mechanoreceptors embedded in the follicle (Fox and Woolsey, 2008). Five types of sensory neurons are known to exist in the follicles of the mouse, Merkel endings, transverse lanceolate endings, longitudinal lanceolate endings, ruffini or spiny endings and reticular endings (Ebara et al., 2002). Little is known about the functions of each of these cell types in the rodent whisker follicle. However, it is clear that mechanoreceptors allow these sensory cells to convert mechanical energy into action potentials, the means of signaling peripheral sensory information to the brain.

A mouse has several (~40) macrovibrissae on each side of its face. They are arranged in a characteristic grid of five horizontal rows, called A-E, and several vertical arcs, named by numbers (Van der Loos et al., 1984; Brecht et al., 1997). There are 4 follicles in rows A & B and 9-12 follicles in rows C-E. Additionally, there is one so called greek arc, where the whiskers are annotated by the first 4 letters of the greek alphabet (α, β, γ, δ, dorsal to ventral). Individual whiskers can be referred to by a letter-number combination (e.g. C2), or by a greek letter. More caudal whiskers are generally longer and thicker. Therefore anterior arcs usually give rise to thin and short whiskers, more posterior arcs longer and thicker whiskers (*Fig.4.1*).
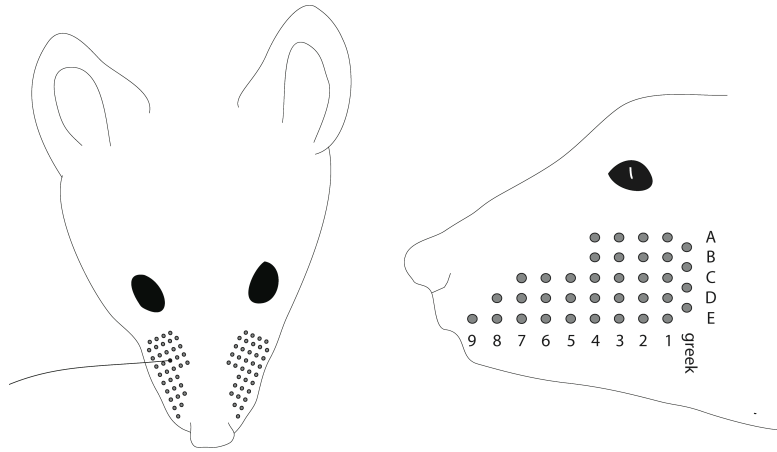
Fig. 4.1: Schematic drawing of the whisker organization on the mystacial pad of a mouse. Five horizontal rows annotated A-E, 4-9 whisker arcs depending on the row. 4 greek whiskers being named α, β, γ, δ from dorsal to ventral.

The muscles in the mystacial region are divided into two categories: extrinsic and intrinsic. Extrinsic muscles move the upper lip, the nose and the whole whisker pad. Intrinsic muscles are attached to the whisker follicles and form a sling connecting two adjacent follicles (***Fig.4.2***) (Dörfl, 1985). These muscles move the whisker in multiple ways (Hill et al., 2008). A change of angle between whisker and whisker pad in the horizontal plane (rostro-caudal) is mediated by the intrinsic muscles and this angle is referred to as azimuth or theta. The change of the follicle position along the rostro-caudal (horizontal) axis of the mouse is a result of extrinsic muscle activity and referred to as translation. The angle between the whisker and the whisker pad in a vertical plane (dorso-ventral) is called elevation, and recently a rotation of the whiskers around their long axis has been reported and named torsion (Knutsen et al., 2008) (***Fig.4.3***).
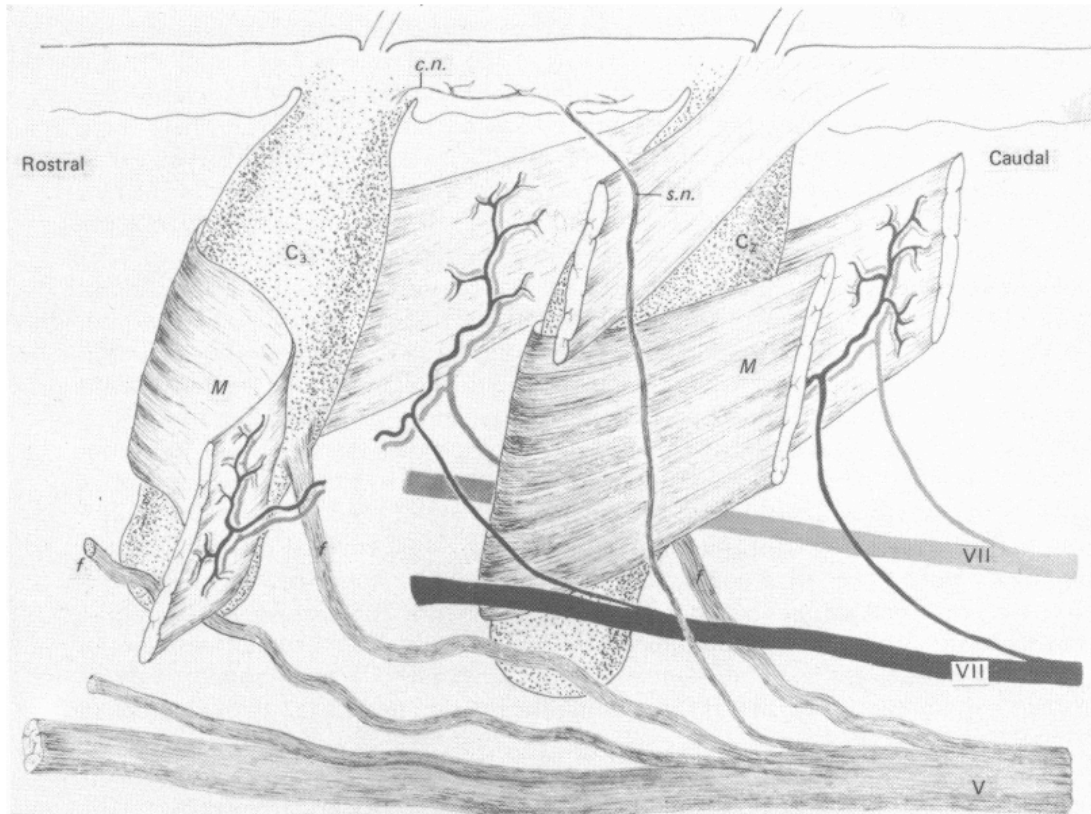
Fig. 4.2: Anatomical drawing {Dörfl, 1985 p01575} showing two adjacent follicles C2 and C3, the intrinsic muscle (M) around C3 has been cut and folded back to show the interfollicular space. Two branches of the facial nerve (VII) target the intrinsic muscles. From the row nerve (V) follicle nerve fibres (f) branch off to target the posteromedial part of the follicles.
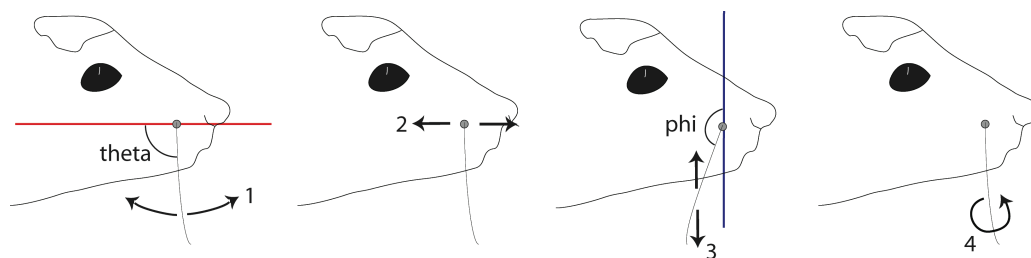
Fig. 4.3: A schematic showing the different modes of whisker motion. From left to right: Arrows (1) indicating azimuth (θ), a movement in the horizontal plane, defined as the angular change between whisker and the rostro-caudal axis (red line). Arrows (2) indicating translation, a movement of the follicle. Arrows (3) indicating elevation (φ) in the dorso-ventral plane (blue). Arrow (4) indicating torsion (ζ), a rotation of the whisker around its axis.

The peripheral branches of the mechanosensory neurons project to the whisker follicle, where they transform the mechanical signals in the follicle into action potentials. These signals are then conveyed through the infra-orbital branch of the trigeminal nerve past the cell bodies which lie in the trigeminal ganglion, via the central branch of the

ganglion cells to the trigeminal nucleus (TN) of the brain stem. The trigeminothalamic neurons in the principal TN are arranged into clusters (barrelettes) representing individual whiskers (Veinante and Deschênes, 1999). The barelettes are arranged into a map corresponding to the grid structure of the follicles seen on the mouse's snout. From this brainstem nucleus three pathways project to higher brain areas. In the best studied pathway, the lemniscal pathway, cells of the principal TN project to and make synapses in the dorsomedial part of the ventral posterior medial (VPM) nucleus of the thalamus, which also shows a somatotopic organization. The anatomical structures representing each whisker in the VPM are referred to as barreloids. The neurons of each barreloid generally respond to deflection of one whisker (the principal whisker) (Brecht and Sakmann, 2002). From the VPM the thalamic cells project mainly to layer 4 of the primary somatosensory cortex (S1). Each thalamic barreloid projects to a cortical barrel in a 1-1 manner. The organization of the cortical barrels resembles almost perfectly the arrangement of macrovibrissae on the snout (Woolsey and Van der Loos, 1970). Therefore, the structure of the whisker organization can be seen in the arrangement of the follicles on the snout, and throughout the stages of the lemniscal pathway, in the barrelletes of the trigeminal nucleus, the barreloids in the thalamus and in the barrels of the layer IV primary somatosensory cortex (barrel cortex) (Petersen, 2007).

The paralemniscal pathway originates from the cells in the rostral part of the interpolar trigeminal nucleus, which is not organized in a somatotopic way. These cells project to the medial sector of the posterior nucleus (POm) and to the zona incerta (ZI). The cells of the POm send axons to layer 5a of S1 and to the primary motor cortex (M1). In contrast to the lemniscal pathway, the paralemniscal pathway does not show the characteristic 1-1 whisker-brain region structure and seems to integrate information from multiple whiskers (Diamond et al., 2008).

4.2. Functions of the Whisker System

Nocturnal rodents, such as mice, use their whiskers to actively whisk against objects in order to locate (Knutsen et al., 2006; Mehta et al., 2007) and identify (Anjum et al., 2006; Ritt et al., 2008; Jadhav et al., 2009) them. Mediated by the extrinsic and intrinsic muscles of the mystacial region, the mice show different types of whisking. These include "exploratory" whisking (Carvell and Simons, 1990; Knutsen et al., 2005) which

is characterized by bilaterally symmetric, high-amplitude movements (rats: 5-15 Hz, mice: 20Hz) and "foveal" whisking, characterized by higher frequency and lower amplitude (rats: 15-25Hz) (Berg and Kleinfeld, 2003a). Rats and mice can also exhibit asymmetric (Mitchinson et al., 2007) and irregular whisker movements when solving a horizontal object localization task (Knutsen et al., 2006; O'Connor et al., submitted). Active whisking is necessary for azimuthal discrimination (Knutsen et al., 2006), but sectioning the facial nerve, thereby impairing the rat's ability to whisk, has no effect on the ability to determine the width of an aperture (Krupa et al., 2001).

To perceive object location, rodents have to define the position of the object along the three axes, rostro-caudal (horizontal), medio-lateral (radial), dorso-ventral (vertical). Most research has been conducted to uncover the mechanisms of horizontal position discrimination. It was shown that rats can reliably detect horizontal location differences between two vertical poles. One vertical metal pole was present on both side of the rat's face and the rats had to judge their relative position (left or right pole being more anterior than the other pole) after aligning their head with a nose poke. After judging, they had to report the position by orienting towards one of the reward dispensing sprouts which were located on either side. Most rats could reliably discriminate differences as small as 6° of azimuthal angle and some even performed well when the difference was 1°. The state of their whiskerfield (either full, arc 2, row C, or only C2) had little impact on their performance. For this task whisking was required, as after bilaterally sectioning the motor nerves performance dropped to chance levels (Knutsen et al., 2006). This task involves comparing the position of two objects relative to each other, but rats also have the capacity to judge position in head centered coordinates without an external point of reference (Mehta et al., 2007; O'Connor et al., submitted).

To find out what signals the neurons of the trigeminal pathway transmit about whisker location, recordings of cells in the trigeminal ganglion (sensory receptor neurons) in artificially induced (electrical stimulation of the facial motor nerve (Berg and Kleinfeld, 2003b)) whisking rats were conducted. Recording has been done both in animals which were whisking in air and when whisking against a vertical pole positioned in different locations.   Three types of neuronal responses could be characterized: "whisking cells", cells that responded to whisking, "touch cells" which fired either upon contact ("contact cells"), sustained pressure ("pressure cells") or detachment ("detach cells"), and "whisking/touch cells" which fired on both instances (Szwed et al., 2003).

Horizontal location could be coded by combining a touch signal, as transmitted by the touch cells, with a signal carrying information about whisker azimuth over time, a temporal reference signal. The firing of the whisking cells in the trigeminal ganglion do in fact carry such information (Szwed et al., 2003). These cells encode the whiskers' position with high precision by firing at specific deflection angles. Therefore, cells receiving inputs from phase specific neurons and touch cells could decode horizontal object position. It has been suggested that these signals converge on cortical cells (Curtis and Kleinfeld, 2006; Kleinfeld et al., 2006), in agreement with observations that rostro-caudal position discrimination is cortex dependent (O'Connor et al., submitted).

The rodents' ability to discriminate vertical locations and neuronal responses carrying this information has been proposed to be based on the row structure of the whiskerfield (Diamond et al., 2008). The mice could monitor in which row a whisker contacting an object is located, which would hold information of the object's elevation.

The discrimination of positions along the third of the three axes, radial distance, has been addressed in an experiment probing the abilities of rats in an aperture discrimination paradigm (Krupa et al., 2001). The rats were trained to align their head between two walls and to judge whether the distance between the two walls was wide or narrow. Rats were able to discriminate width differences of as little as 3 mm. In this task rats did not actively whisk against the walls and abolishing whisking by bilaterally sectioning the facial nerve did not impair performance, as rats contacted the walls with their whiskers through head and body movements. Integrating information over multiple whiskers seems to be necessary for aperture width discrimination: Performance dropped correlated with decreasing number of whiskers used, as seen when progressively trimming the whiskers. Accordingly, performance dropped to chance levels when rats were left with one whisker on each side of the face. The task is cortex dependent.

There are however some limitations of the experiment in its contribution to characterize perception of radial position of objects. As the whisker-movement behavior of the animal was not controlled or monitored, it is hard to judge how the animals actually solved the task. When allowing free movement, the rats can solve the task also with

azimuthal cues not using radial cues which makes it safer to speak of the task as an aperture discrimination task rather than a radial distance discrimination task.

It has been proposed that rate-of-change of moment could be used as a prominent mechanical cue to judge radial distance (Birdwell et al., 2007). As the whiskers are tapered rods, the farther lateral the stimulus contacts the whisker, the more the whisker bends and the less rapid is the change in moment applied to the follicle. Moreover, the majority of touch cells in the trigeminal ganglion show an increasing firing rate the smaller the radial distance between follicle and object becomes. This firing property leads to a higher number of active touch cells, additionally recruiting touch cells with high spiking thresholds (Szwed et al., 2006). Upon this observation, it was proposed that radial distance can be encoded by firing rate or spike count.

4.3. Experimental Rational

One of the major goals of systems neuroscience is to dissect the mechanisms underlying the processing of sensory information in neuronal circuits and how circuits mediate behavior. Recent advances in the design of genetically encoded molecules to measure activity and to control the activity of subsets of neurons provide powerful tools for this line of research (Luo et al., 2008). Expressing these proteins in genetically defined cell types may allow a reproducible way of characterizing the function of neuronal populations. Therefore, these tools are most powerful when applied to genetic model organisms such as the mouse (O'Connor et al., 2009), for which an abundance of tools for targeting neuronal subpopulations and tracing of neural connectivity exist.

Analysis of neuronal circuits requires experiments in behaving animals. When wanting to work on the mechanisms of behavior it is no surprise that non anesthetized animals have to be the model of choice, but the same applies to all research of cortical information processing, as cortical neural network dynamics are fundamentally different in awake and anesthetized animals. Cellular physiology techniques, including whole cell recording and two photon imaging, require stable recording setups. Even though it has been shown that both imaging and whole cell recordings can be done in freely moving animals (Lee et al., 2006; Helmchen et al., 2001), these techniques are laborious or not applicable to mice. Head fixation constitutes a good solution permitting

the work on awake behaving animals in a stable setup and providing excellent behavioral control and repeatability across trials.

Both of the last mentioned points are absolutely critical. Repeatability of trials allows to acquire recordings of neuronal activity and corresponding behavior over large numbers of equivalent trials. This makes it possible to uncover subtle quantitative relationships between neural activity and behavioral variables. The second point, the behavioral control, is important to be able to make correlations between behavior and neural activity with high resolution, therefore with many discrete behavioral variables.

A somatosensory behavioral task was developed in the Svoboda laboratory which renders it possible to meet these requirements (O'Connor et al., submitted). Head fixed awake behaving mice have to discriminate the positions of a pole, which is dropped within reach of their whiskers. High speed video recording and subsequent tracking of the whiskers yield information about active touch and sensation. Recording of mouse's licking behavior (a trained behavior, reporting position perception) which follows a position judgment with high temporal precision gives knowledge about behavioral output.

But the knowledge of the external variables such as whisker position, speed, curvature, etc. is not enough, it is also necessary to know which of these variables are relevant to the mouse for solving a specific task. This has been done to some extent on the question how mice detect differences in anterior-posterior position of an object (Mehta et al., 2007; Knutsen et al., 2006)(O'Connor et al., submitted) but empiric information on the mechanic variables used by the mice to judge radial distance is lacking. To answer questions on how the brain constructs a representation of location it is necessary to know what input variables for every dimension of space are used. Neither is it known whether mice can discriminate differences in radial distances, nor which strategies they prefer to do so, whether they can do the discrimination with non-azimuthal cues and which mechanic variables they use for pure radial distance discrimination. This study set out to answer these questions.

5. Materials & Methods

5.1. Materials

5.1.1. Mice

The 8 mice used for the following experiments were adult, therefore older than 60 days after birth (>P60), males of the C57BL/6Crl strain (Charles River, MA USA). Their age when ordered was 3 weeks. Mice were housed individually in cages containing tunnels and bedding material, in a reverse light cycle room that was dark from 9 am - 9 pm. The weight and health of the mice was monitored daily by the Janelia Farm vivarium staff and all procedures were in accordance with protocols approved by the Janelia Farm Institutional Animal Care and Use Committee.

The animals will be referred to with their identification numbers JF25395, JF25396, JF25397, JF25398, JF25399, JF25400, JF25401, JF25402, JF25403. Each mouse of the original batch of 9 mice was used throughout all of the experimental time, except JF25398, which died shortly after arrival during the period of adaption to the vivarium and was therefore never used for training or experiments. In the following table the participation of each individual mouse in the conducted experiments is listed.

Mouse	Discrimination Training	Whisker length	Psychometric Curve	Fooling	Fooling control	Control
JF25395	✓	X	✓	✓	✓	X
JF25396	✓	✓	✓	X	X	X
JF25397	✓	X	X	✓	✓	X
JF25398	X	X	X	X	X	X
JF25399	✓	X	X	✓	✓	✓
JF25400	✓	✓	✓	X	X	X
JF25401	✓	✓	X	✓	X	✓
JF25402	✓	X	✓	X	X	X
JF25403	✓	✓	✓	✓	✓	X

Table 5.1: Table shows whether each mouse (rows) participated (green tick) or didn't participate (red x) in the conducted experiments (columns).

5.1.2. Experimental Control

For the behavioral experiments two computers controlled several pieces of equipment, including motors, valves and sensors in real time.

One computer running the MATLAB behavior software (see Methods section), transfered all necessary information for the current trial to the Super Logics Computer via an Ethernet connection. Between the trials it sent commands to the controllers of the stepper motors, which then in turn drove the motors.

The Super Logics (Waltham, MA USA) computer allowed real time control of the experiment. The computer received information from the MATLAB computer between trials and then executed all commands necessary for a current trial with high temporal precision. Connected to the peripheral devices via the break-out box and receiving information of the mouse's behavior via its data acquisition board, this computer was used for controlling all devices relevant for the behavior during the trials.

The break-out box (Island Motion Corporation, Long Island, NY) receives inputs from the Super Logics computer via a National Instruments connection and passes on the received signals for the Festo valve (resulting in moving the stimulus) and the air puff valve to the Air-Valve Control Box. The signal for the camera trigger is transmitted to a National Instruments external camera board which in turn passes the signal on to the Imaging Computer (see below).

The air-valve control box was custom designed and constructed by Dan O'Connor to receive 5V logical inputs from the break out box and to transform the inputs to higher currents in order to open the connected valves. The box is transmitting a high power signal to the air puff valve (see below) and a 24V signal the Festo solenoid valve (see below).

5.1.3. Behavior Rig

The behavior apparatus was mounted on a vibration-isolation table and enclosed in a custom-built light-isolating box. The interior of the box was lined with sound-isolating foam (McMaster, P/N: 5692T49) and the front side of the box was covered with a light-isolating curtain (Thorlabs P/N: BK5) lined with sound-isolating foam (McMaster, P/N:

5692T49). Mice were monitored with a small infrared-sensitive video camera (Super Circuits, P/N: PC212XS), for which illumination was provided by the scattered light of the light source for high speed videography (see below). Various mounts from Thorlabs were used to mount the mouse and devices.

5.1.4. Head Fixation

For head-fixation, a custom-designed and machined titanium head post was mounted to the skull of each animal (for procedure see methods section)(*Fig.5.1*).
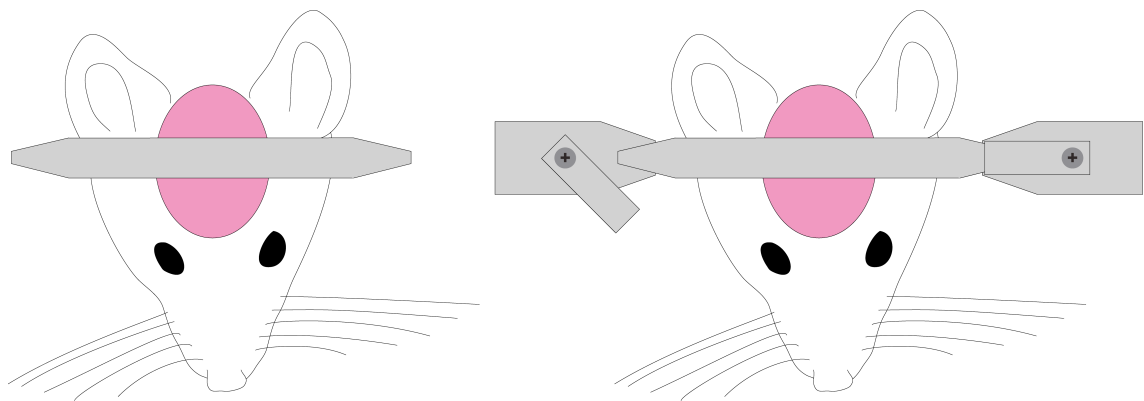

Fig. 5.1: Left drawing showing the design of the head post. Head Post in grey, dental acrylic in pink. Right drawing showing the head post being fixated in the head post holder. For purposes of intelligibility the left head post holder is not shown in a tightened state.

5.1.5. Movement Setup

The entire movement-assembly was mounted on a pneumatic linear slider (Festo SLS-10-30-P-A Mini slide, P/N: 170496). This pneumatic system rapidly brought the stimulus into and out of reach of the whiskers, triggered by a computer controlled solenoid valve (Festo CPE 10-M1BH-5L-QS-6, P/N: 196883) and connected to a compressed air source.

To move the pole into the desired positions within the whisker field, two stepper motors (Zaber, P/N: NA08B30) with submicron resolution (10499 micro steps = 1mm) were used. One of them was mounted with the custom designed aluminum mount # 1 directly to the Festo pneumatic slider. The second one was mounted with the custom designed mount #2 to a stainless steel linear slider (Schneeberger, P/N: NDN 2-50.40) which was also directly attached to the Festo pneumatic slider via mount #1 and moved by the first motor. The second motor itself also moved a second Schneeberger slider, to

which the pole was attached (see below). This setup, allowing the first motor to move the second motor, gave the opportunity of 2D-control of pole position.

5.1.6. Stimulus Assembly

The actual stimulus in form of a pole was held by a custom designed and machined titanium pole holder (***Fig.5.2***), which in turn was mounted on the second Schneeberger slider. For the flexible object, a second pole holder was glued to the main pole holder. The stimulus dropped into the whisker field of the mouse's right cheek.

Three types of objects have been used for the experiments. A stainless steel dowel pin (McMaster, P/N: 90145A427) with a diameter of ~1.59 mm (1/16 inch) and a length of ~3.8cm (3/2 inches) was used for training and experiments investigating the length-dependence of whiskers for radial object localization. For all the other experiments a thinner pole (diameter 0.4 mm) was used. For fooling experiments a flexible object had to be used in a subset of the trials. The flexible object was a cleaned and straightened rat's whisker, which was glued to a metal pole. Three of these flexible objects have been used, which differed in their mechanical stiffness.

For the fooling experiment both the thin steel pole and the flexible object had to be used interchangeably within one session. Therefore the steel pole was mounted on the pole holder as always but a second custom built holder was glued to the original pole holder to keep the flexible object in place (***Fig.5.2***). The distance between steel pole and flexible object was defined in a way that when the steel pole was dropped into the whisker field the flexible object was in front of the mouse and out of reach. In trials when the flexible object dropped into the whisker field, the steel pole was out of reach of the whiskers.
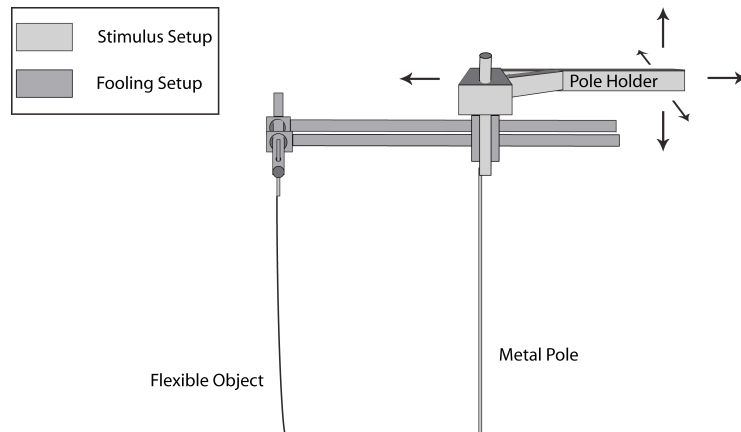
Fig. 5.2: Schematic showing the standard stimulus assembly of pole holder and the thin pole (metal pole) in light grey. The mouse's snout would be on the left, dorsal is up. Second pole holder to keep the flexible object in place is shown in dark grey and is mounted on the pole holder. The distance between the metal pole and the flexible object could be altered.

5.1.7. Reward and Punishment

A custom acrylic lickport, used to record licks and to deliver water rewards, was placed on a manipulator (Newport, P/N: MT-XYZ) in front of the mouse, within reach of its tongue. Licks were recorded as interruptions in the light path between an 860 nm LED (Digikey, P/N: LN77L-ND) and a phototransistor (Mouser Electronics, P/N: 512-L14G1). To maximize free space around the face for high-speed videography, the phototransistor/LED pair were remote from the lickport but coupled to it using a 1 mm acrylic fiber optic (Edmund Optics, P/N: NT02-544). The phototransistor and LED were paired with a custom made circuit board (Island Motion Corporation, Long Island, NY), which signaled through analog signaling (voltage drop) to the data acquisition board in the Super Logics computer. The Super Logics computer sampled the voltage with a frequency of 6 kHz and depending on the trial type and on the state of the trial a reward or punishment was delivered via the break-out box or nothing happened.

Water for rewards was delivered by gravity into the lickport through a 1.3 mm (1/20 inch) diameter steel tube, under solenoid valve control (The Lee Company, Westbrook, CT). In order to limit the amount of time water remaining at the lickport and to prevent pooling, excess water was pumped out of the lickport through a 0.0325" tube using a peristaltic pump (Rainin Instrument Co Inc., Rabbit Plus peristaltic pump)

Punishment was provided by puffs of compressed air (typically 10 psi) delivered through a small metal tube (~2.3 mm inner diameter) pointed at the face from a

distance of several centimeters. The punishments were gated by a solenoid valve (The Lee Company, Westbrook, CT) connected to a compressed air source. The solenoid valve was under electric (high power) control of the Air-Valve Control Box which delivered the signal for the valve to open when receiving a respective signal from the Super Logics Computer with the same mechanism described for reward delivery.

5.1.8. Imaging

To measure the movement of whiskers during behavior, a high speed videography system was set up. This included a monochrome digital high speed CMOS Area Scan Camera (Basler, P/N: A504 K) which was linked to an imaging computer. When recording, the frames were directly written on the hard drives of the RAID in the imaging computer. The recording was triggered by the Super Logics Computer via the Break Out Box and connected to the latter one, a National Instruments external camera board (P/N: BNC-2090A) which passed the signal on to the Imaging computer. The imaging software (Norpix, Streampix 3) allowed the automatic recording of videos during the session.

In order to image the whiskers without the mouse being able to see the pole, infrared light was used to illuminate the whiskers. A high power LED (Roithner Lasertechnik, P/N ELJ-940-211) emitting light with a wavelength of 940 nm illuminated the whiskers from above the mouse. The light was delivered through a diffusor and a condensor lens and the light path was pointed directly into the camera lens with a mirror (Thorlabs, P/N: ME2SG01).

5.1.9. Training Rigs[1]

As early training does not require precise definitions of positions and no imaging, the training process was sped up by using three training rigs. These rigs were set up in a way that three mice can be trained in parallel and are built similarly to the main experimental rig. The main difference is that the pole can only be moved along the anterior posterior axis, as only one motor is being used. Also a different valve (Nresearch P/N: HP225T011) to control air puff delivery was used and the rigs did not include a whisker imaging setup.

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[1] Training Rigs were set up by Daniel O'Connor.

5.2. Methods

5.2.1. Head Post Surgery [2]

Head fixation required implantation of a small custom built titanium headpost that could be secured to a mount in the behavioral rig (***Fig.5.2***). Mice were anesthetized with isoflurane (~1.5-2% by volume in O_2) and were unresponsive to tail pinches. The eyes were covered with a thin layer of petroleum jelly. Bupivacaine or lidocaine HCl (10 μl, 0.5% S.C.) was applied locally at the incision site. Mice were mounted in a stereotaxic apparatus (Stoelting, Chicago IL) and kept on a thermal blanket to maintain body temperature. The scalp and periosteum over the dorsal surface of the skull were removed. A thin layer of cyanoacrylate adhesive was applied to the skull and covered with dental acrylic (Lang Dental Jet Repair Acrylic 1223, Henry Schein P/N: 1251546). Headposts were fixed to the skull using dental acrylic. Buprenorphine HCl (0.05-0.10 mg/kg I.P., Bedford Laboratories) was used for post-operative analgesia. Ketoprofen (5 mg/kg) was used at the time of surgery and post-operatively to reduce inflammation. On the day of surgery mice had not yet been water restricted, and were allowed 10 days to recover prior to being put on the 1 ml/day water regimen described below.

5.2.2. Head Fixation

Mice were placed in acrylic (2.9 cm (1-1/8 inches) ID; McMaster P/N: 8486K433) tubes such that their heads extended out the front and they could use their front paws to grip the tube edge. The headpost was secured in the headpost holder guaranteeing the head to be in the same position over all sessions. The procedure was done prior to each session without the use of anesthetics. Mice were thereby head-fixed in a natural crouching position with their whiskers free to move around the space surrounding their heads.

5.2.3. Water Restriction

For 10 days prior to training and on days without behavioral testing, mice were maintained on 1 ml of water/day. On days with behavioral sessions, mice generally obtained all water for the day during the session and were allowed to perform until sated. The amount consumed was determined by weighing the mouse before and after the session (including any excrement). The volume consumed, often totaled less than

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[2] Head post surgeries on all eight mice were performed by Daniel O'Connor.

or more than 1 ml, and was determined largely by how much water the mouse had consumed the prior day. Only mouse JF25395 was kept on 1.5 ml/day to maintain its weight above a threshold. The weight and health of the mice was monitored daily, to prevent a drop below 70% of pre-restriction weight. All procedures were in accordance with protocols approved by the Janelia Farm Institutional Animal Care and Use Committee.

5.2.4. Whisker Trimming

For all experiments shown except the one to address the whisker length dependent strategy (see below) the whisker fields of the mice were trimmed in a way that only the principal[3] whisker C2 could contact the pole. This is necessary to ensure, that the azimuth at initial contact (first moment when contact between whisker and pole is established) does not differ between the go and the no-go position which would not be possible when multiple whiskers with different positions on the whisker pad contact the object. Only the whisker field on the side of the stimulus (right side of the mouse) was trimmed. Depending on the whisking behavior of the individual mouse this meant either trimming all whiskers except C2, when the mouse was whisking strongly, or additionally leaving Greek whiskers δ and γ. The trimming process stretched over 12-27 days, whereby the more caudal whiskers were cut first, gradually leading to a situation where the mouse only used the principal whisker to contact the object. The trimming process was deliberately stretched out to allow the mice a slow adaption to a greatly reduced whisker field, in an attempt to minimize the risk of a loss of the principal whisker. Whiskers were trimmed every 2-4 days.

5.2.5. Matlab Codes of Behavioral Paradigm

The entire apparatus was controlled by a software system, initially developed at Cold Spring Harbor Laboratory (Z. Mainen, C. Brody), comprising a MATLAB (Mathworks, Natick MA) package on Windows XP communicating over the ethernet with a hard real-time control system implemented in C on Linux using the RTAI (www.rtai.org) kernel patch (C. Culianu, Cold Spring Harbor Laboratory). The real-time Linux system interfaced with valves and the camera trigger and recorded licking responses.

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[3] The term "principal whisker" as used from here on, is defined as the single whisker, the mice were able to use for contacting the stimulus. C2 has been chosen as the principal whisker.

In our behavioral paradigm the mice were head-fixed and in every trial a metal pole was descending (descending time ~270 ms) in one of two user defined positions. Upon presentation of the pole the mice had to judge the position of the pole using their whiskers and then had to decide on a response. One position was defined as a go position, which meant that upon licking and breaking the beam of the lickport the animal was able to actively trigger a water reward (scored as a "hit"). The other position was defined as a no-go position, which meant that upon licking and breaking the beam (a "false alarm") an air puff was delivered as a mild punishment. A time-out of 2-10 seconds was introduced as an additional punishment. The exact length of the time out could be changed during the session over the GUI. No licking was neither rewarded nor punished in both go ("miss") and no-go ("correct rejection") trials.

The licking triggered the reward or punishment only after a certain time period after the start of the trial, defined as the sampling period time or grace period, and had to occur within a certain time defined by the answer period time. In case of no beam break in the response window between the end of the grace period and the end of the answer period time, the trial would end and the pole ascend (pole ascending time ~270 ms). The sampling period time could be altered during the session via the GUI, the answer period time was typically 2 seconds. In case of a hit the water valve would open for a time period (water valve time), which could also be defined during the session over the GUI and was typically 0.06-0.11 seconds long. After the opening of the water valve a time period (drinking time) was granted, allowing the mouse to finish drinking before the end of the trial (*Fig.5.3*).
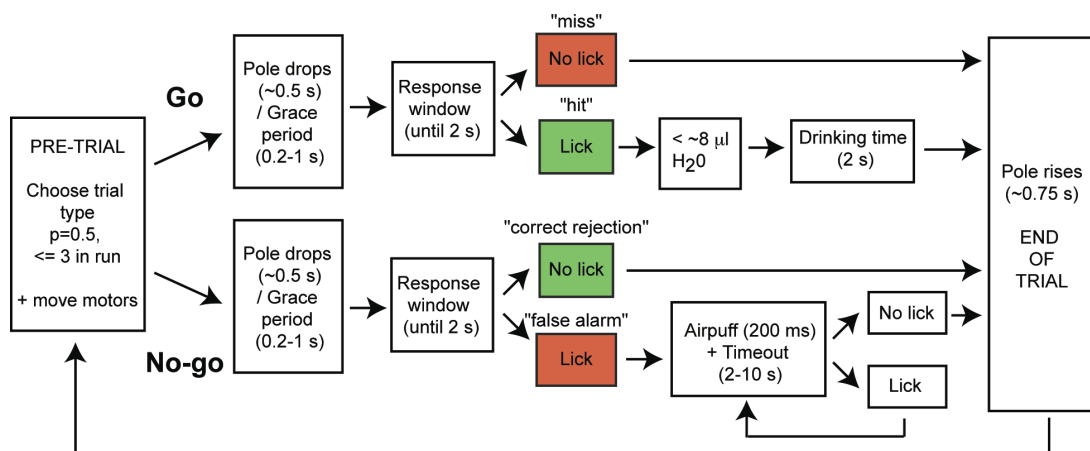

Fig. 5.3: Graphical depiction showing the organization of the behavioral paradigm. In green correct response, in red erroneous response for corresponding trial type. From (O'Connor et al., submitted).

I adapted the task further, which was designed for azimuthal (rostro-caudal direction) distance discrimination, by writing functions which chose positions differing in radial distance (medio-lateral direction) for one whisker. The specific adaptions varied between the experiments and will be treated separately for every experiment in the descriptions of the experiments below. The Matlab codes enabling the original program to execute the necessary commands can be found in the appendix (*Chapter 8.1*).
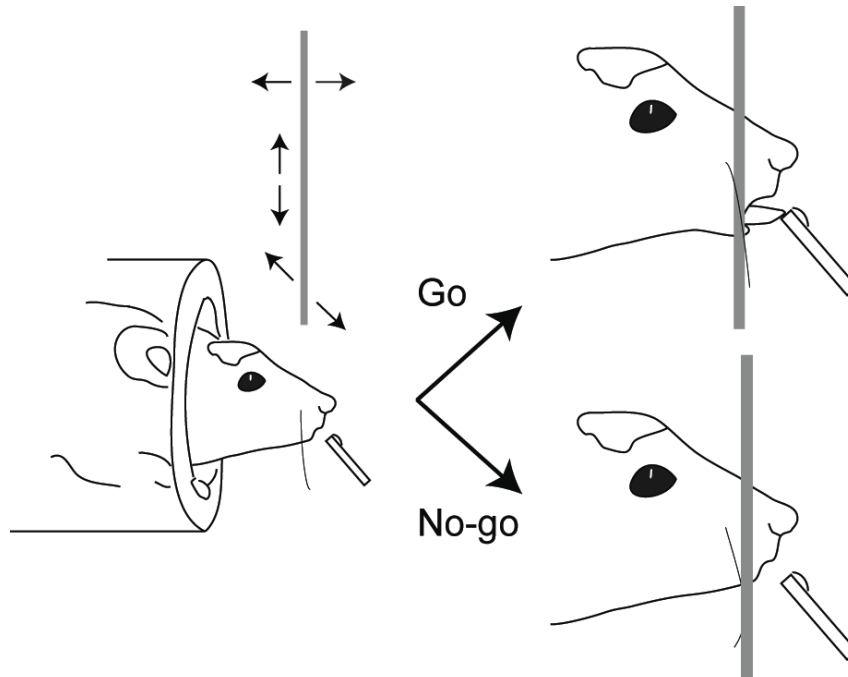

Fig. 5.4: Graphical depiction of the discrimination task. The left side shows the inter-trial period in which the pole is out of reach and is moved by the motors into position for the next trial. The pole is then brought into the whisker field by vertical movement mediated by a pneumatic system. Once within reach of the whiskers the mice judge the radial distance of the pole and respond accordingly; by licking in the go position (proximal) and withholding licking in the no-go position (distal).

The probability of the two trial types was usually 50%. To prevent discouraging mice with a succession of no-go trials, the number of successive trials of one type was limited to three. Both the probability and number of successive trials was kept constant.

In order to prevent mice from using auditory cues to solve the task, the motors did not move directly to the position of the next trial but first to an intermediary position and only afterwards to the final positions. The intermediary positions were defined differently for every experiment and were chosen to diminish or greatly complicate differences in motor movement depending on trial type. For all experiments in which

the mice had to discriminate between two positions (one go position and one no-go position) the 2-radial positions function[4] was used, which defined the intermediary position as the midpoint between the go and no-go position. For the training task with a range of no-go positions[5] the intermediary position was randomly chosen for every trial from all possible positions on the line between the go position and the most distal no-go position. For the experiments with a range of no-go positions and the flexible object[6] (fooling experiment and its control[7]) the intermediary position could either be a position randomly chosen between the go position and the most distal no-go position or the coordinates used for positioning the flexible object in the desired location. Although as the motors were required to make large movements to reach the position of the flexible object, which took up to 4 seconds, only in 20% of the trials was the flexible object position chosen as the intermediary, in an attempt to reduce the mouse's waiting time and the overall session time.

5.2.6. High Speed Videography

High speed video recordings provided accurate information about the whisking behavior of the mice when solving the task. A bottom view of the mice was recorded, which featured the right side of the mouse where the stimulus was presented and covered a field of view including the entire principal whisker (C2) up to the midline of the mouse's head. The frame size was 310x200 pixels in the middle of the camera's chip corresponding to ~2.2x1.4 cm in the plane of the principal whisker (1 pixel ≈ 0.0719 mm). Videos were recorded either with 500 frames per second (fps) or 1000 fps, with an exposure time of 0.2 ms. For every trial a movie of 1.5 seconds length was recorded, starting 83 ms before the triggering of the pole descent.

5.2.7. Whisker Tracking

In order to be able to make quantitative arguments about whisker properties during the contact, the principal whisker C2 was tracked in a subset of trials in the radial distance

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[4] For the code see appendix 8.1.1.

[5] For the code see appendix 8.1.2.

[6] For the code see appendix 8.1.3.

[7] For the code see appendix 8.1.4.

discrimination experiment and the fooling experiment. To do so, a custom written software[8] in C and Python was used.

A primary design goal for the algorithms was scaling to large data sets where comprehensive human validation is not feasible and where computation time must be considered. For example, video images corresponding to a single behavioral session can amount to more than one million images. In addition, during the object localization task mice palpated the pole extensively; the whiskers often underwent large changes in curvature (>0.25/mm) and rapid motions (>10000°/sec angular velocity). Thus, determining shape and position of individual whiskers was more challenging compared to previously treated experimental situations where animals whisked in air or lightly against surfaces. Our method involves several steps: 1) pre-processing; 2) detection of whiskers; 3) tracing of whiskers; 4) determination of the identity of traced whiskers.

1) Pre-processing. For the images collected as part of this study it was necessary to correct images for a systematic multiplicative bias (2-3%) between neighboring pairs of scan lines introduced by the high speed camera. The ratio of the intensity between a pixel and an above neighboring pixel was computed for each pixel in odd-numbered horizontal lines across a movie. The bias was then measured as the mean of these ratios for pixels within an intensity range chosen to avoid quantization and saturation errors. These were the pixels with intensities above the image mean (150 to 160 typically) and 2% below saturation (250). Multiplying evenly numbered lines by the measured bias corrected the image, improving downstream analysis.

2) Detection of whiskers. Initiating sites were found by analyzing the locations of local minima. Along whiskers local minima lie along a line, whereas elsewhere they do not. In a 5x5 window about each pixel, local minima were located and the principle direction of their positional variation determined. Computing the fraction of the total variance lying in the principle direction and applying a threshold resulted in 50-100 initiation sites were found per whisker, with 10-20 false positives per image. The threshold was determined empirically by maximizing true positives while minimizing false positives over a representative subset of 10 images. Whiskers were always detected. Each image was analyzed independently to find initiating sites. Alternatively, the initiation site may be chosen manually using a graphical user interface.

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[8] Whisker Tracking Software developed by Nathan Clack and Eugene W. Myers in Janelia Farm, description of algorithms by Nathan Clack from (O'Connor et al., submitted).

3) Tracing of whiskers. Tracing was started at the initiation sites, removing them as they were traced over. On average, 1.3 traces were initiated per visible whisker. Duplicate traces sometimes occurred and were merged by averaging. Tracing proceeds by estimating the whisker angle locally, and then bidirectionally extending the trace from that point until both ends terminate. The output is a sequence of positions distributed along the center of the whisker.

Tracing is based on a whisker modeled as a rectangular valley in the image, with variable position, width and angle. The center of the whisker is estimated with sub-pixel precision by finding a position that minimizes the Laplacian of the correlation between the model and the image, or correlating the Laplacian of the model (the line detector) with the image. Approximating the Laplacian of the model, a line detector was designed as two rectangular, identically oriented, step-edge detectors (0.8 mm long, typically 1 pixel (40 μm) wide) positioned parallel to one another and spaced by the detector width. The length was chosen to match the expected distance over which highly curved whiskers remained approximately linear. To evaluate the correlation at a given point, it was necessary to compute a pixel representation of the detector by evaluating the area integral of the detector over each pixel's square domain. The value of the correlation at that position is then the dot product between pixels in the image and pixels in the evaluated detector. For efficiency, discrete representations of the detector were pre-tabulated.

From an initial starting point and angle, traces were extended by making small (40 μm) steps in the direction indicated by the angle. At each step, a steepest descent method was used to optimize correlation with the detector as a function of whisker width, angle and sub-pixel offset. This was performed over a bounded, discrete parameter space with 2.5° angular precision and a positioning precision of 0.1 pixel (4 μm). Width was estimated to 0.5 pixel (20 μm) precision.

Tracing was stopped if correlations dropped below threshold. Additionally, tracing was stopped if one of several criteria indicated the optimization procedure could not be trusted to give an accurate representation of whisker shape beyond that point. This was necessary to handle cases where whisker segments crossed or were partially occluded by the stimulus. The tests were for large angular change between steps, large left-right asymmetry in the intensity field about the detector, or low mean intensity about the detector. If one of these tests failed, several one pixel steps would be taken

along the last trusted direction. If tests and correlations were satisfied at one of these points, normal tracing would resume. Otherwise, the trace was terminated at the last trusted point. Traces shorter than 2 mm were classified as hairs and rejected.

4) Determination of the identity of traced whiskers. While the current implementation of the tracker automatically identifies whiskers (Clack et al., in preparation), for this study whisker identity was established using the Python-based user interface and semi-automated specification; every video frame was viewed to confirm proper tracing and to establish whisker identity.

5.2.8. Training

The goal of the behavior was to establish a robust readout of the mouse's perception of object location in its whisker field, which could be learned quickly by the mice.

After the 10 days of recovery from the head post surgery and the 10 days of water deprivation, the mice were started to be trained in the training rigs, with the following protocol.

Day 1. On the first day, the mouse has to learn to associate the lickport with the water reward. Therefore, as soon as the mouse is extending its tongue, the beam break triggers an opening of the water valve. There is no trial structure and no pole movement and the mouse continuously licks until sated.

Day 2. On the second day of training, the water flow can only be triggered by the tongue if the pole is descended into the whisker field. By moving the lick port away from the mouse, licking becomes more laborious for the mouse which results in less compulsive licking. That way the mouse learns to associate the pole with a water reward. There are no no-go trials and no punishment at this stage of training.

From Day 3. On day 3, the actual task of position discrimination is introduced. The pole drops either in the go (posterior) or in the no-go (anterior) position. The water reward is only available in the go position. The mice tend to lick as soon as the pole drops and the challenge is to train them to withhold the licking response in the no-go trials. The punishment for licking in no-go trials (false alarm) is usually not introduced until after around 10 days of training but varies depending on the individual mouse's

performance. The distance between go and no-go is gradually reduced with the performance increase of the mice, starting off with 8.6 mm down to 2.4 mm.

As soon as the mice performed reliably (>85%) in the 2.4 mm anterior-posterior position discrimination task they were transferred to perform on the behavior rig, where high speed video recording and defining of positions along two axes was possible. There the task was switched to a medio-lateral discrimination task, the go position being more medial than the no-go position. The mice were trained to discriminate distances of 8 mm - 3 mm with a full whisker field and generally as their performance at 3 mm discrimination surpassed 90% the whisker field was gradually trimmed. The mice were divided into two groups of 4 mice each, with the group designated for the whisker length dependent experiment undergoing a well defined trimming process (see below). The whisker trimming of the remaining 4 mice is described in the whisker trimming section in the Methods. After the whisker trimming the experiments were conducted.

5.2.9. Specification of Object Position

If one wants to do experiments characterizing radial distance discrimination, it is very important to define the go and the no-go positions in a way that the azimuth of the whisker at initial contact is the same for both positions.
At first an azimuth at which the contact with the pole should occur was chosen by looking at recorded movies of the whisking animal. Then the position of the follicle and a point along the whisker shaft at this azimuth were defined in pixel coordinates in the frame. By moving the pole directly above the defined positions in the frame, the follicle position and the point along the whisker were defined in motor coordinates. As the point of origin and the direction were known, the go position was always defined as 5 mm from the follicle in the defined direction of the contact azimuth.

For all experiments except the fooling experiment and its control, additionally to the go position one no-go position was defined along the same direction vector from the follicle with a distance that depended on the requirements of the experiment. For the experiments using a range of no-go positions a medial border and a lateral border of a no-go range were defined along the direction vector and for every no-go trial a position between these two borders along the direction vector was randomly chosen.

5.2.10. Strategies for Radial Distance Discrimination with Multiple Whiskers

Four mice (JF25396, JF25400, JF25401 and JF25403) were used for this experiment to test the importance of whisker length differences and multi whisker integration for radial distance discrimination and to characterize preferred radial distance discrimination strategies. After performing at high levels at a discrimination of 3 mm in radial distance with a full whisker field, the mice's whiskers were trimmed for the experiment. All whiskers of arc 3 and more rostral were cut close to the follicle, the same with all other whiskers which were either too short or too vertical to touch both positions. These would contact only the more medial (go) position and could therefore not be used for this experiment. This was typically α and A1 (see the table below for exact listing of whisker trimming for every mouse). In arc 2 the principal whisker C2 was not trimmed and whiskers B2 and D2 were trimmed to a length that allowed them to only contact the 1.5 mm diameter pole in the go position but not in the no-go position. E2 and A2 were cut close to the follicle. The rest of the whisker field (greek whiskers (except α), B1, C1, D1 and E1) were typically not trimmed and could also contact both positions. All in all 6-8 whiskers could contact both positions and 2 whiskers could contact only the go position, whereas the positions were chosen in a way that the azimuth of C2 at contact was the same for both go and no-go position.

√ -not cut	A1	B1	C1	D1	E1	α	β	γ	δ
JF25396	x	x	√	√	√	x	x	√	√
JF25400	x	√	√	√	√	x	√	√	√
JF25401	x	√	√	x	√	x	√	√	√
JF25403	x	√	√	√	√	x	x	√	√

Table 5.2: Table showing the state of the whisker field of every mouse used in the experiment. Green ticks meaning remaining whisker, red x for cut whiskers.
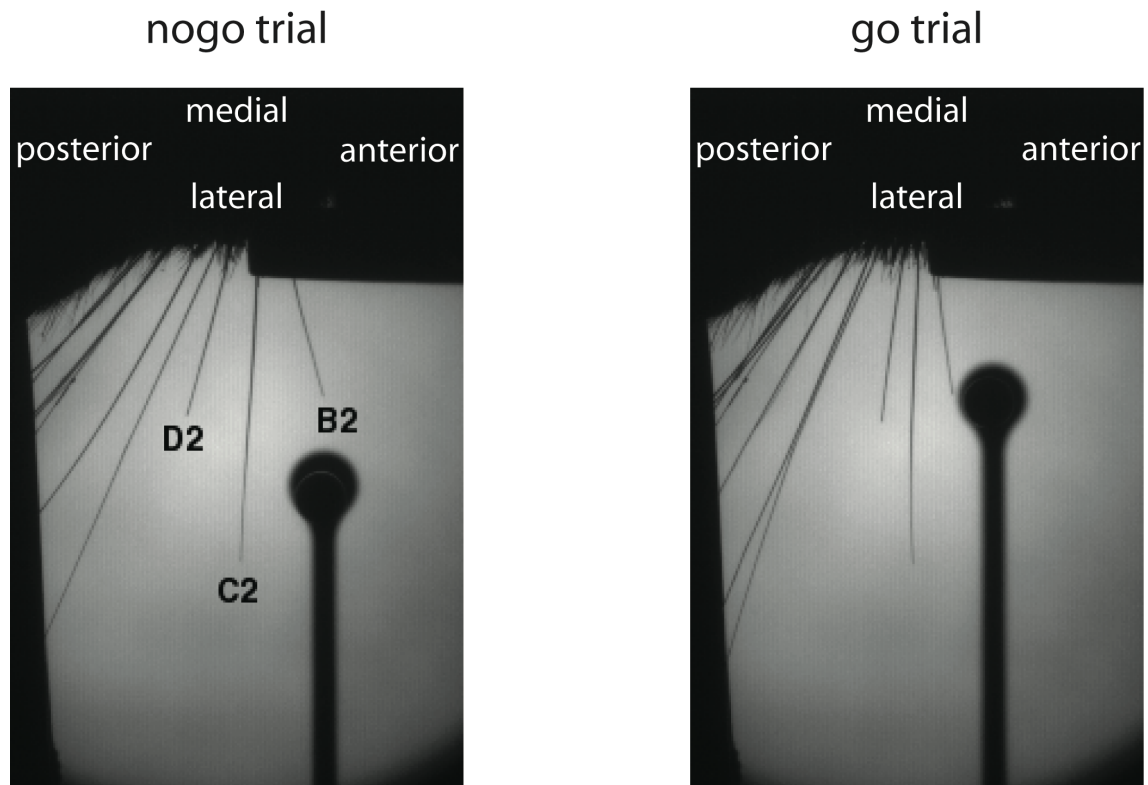
Fig. 5.5: Two frames showing the whisker field of JF25396 from below after the first stage of whisker trimming. Left image - no-go trial, right image - go trial. B2 & D2 can only contact the pole in the go position. C2 and the more caudal whiskers can contact the pole in both positions.

The animals were then trained on a 3 mm radial distance discrimination task, and as soon as the mice's performance crossed 85%, they were transferred to the next stage of the experiment. If the mice didn't reach the 85% threshold after 8 days, they were also transferred to the next stage. For this second stage of the experiment one of the two short whiskers (D2) was cut close to the follicle. This procedure (~5 minutes) was performed under anesthesia (isoflurane ~1.5-2% by volume in O_2) prior to the experiment and after the trimming the mice were given a 15 minute recuperation time in their cage in the dark, after which the impact of losing D2 on the performance levels was tested by running them on the discrimination task.

On the following day, again prior to the experiment and following the same protocol as the previous day, the remaining short whisker (B2) was cut close to the follicle and the effect of the loss of B2 on the discrimination performance was studied by running the animals on the behavioral task after the recuperation period. On all days of the experiment the positions were held constant.

5.2.11. Radial Distance Discrimination Experiment

The goal of this experiment was to see whether mice are able to discriminate positions along their whiskers (radial distance discrimination) by measuring forces in the follicle without azimuthal cues. Therefore the positions were carefully chosen in a way to minimize azimuthal differences of the principal whisker at initial contact (first moment of contact when whisker touches the pole) for both go and no-go positions.
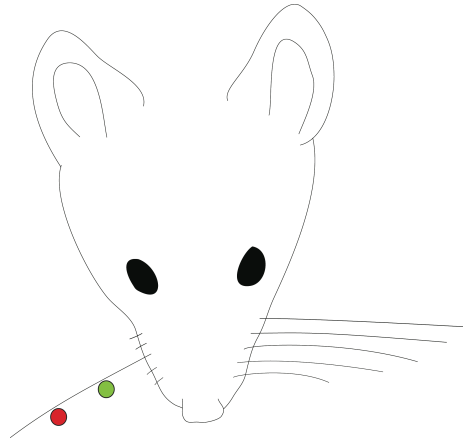

Fig. 5.6: Drawing showing the radial discrimination task, with the go position (green) and the no-go position (red).

Then the mice were trained to reach performance levels of >90% for at least 100 consecutive trials at a 3 mm discrimination task. All 8 mice were participating in the experiment with a whisker field that only allowed the principle whisker to contact the pole (see whisker trimming procedure above). The 0.4 mm diameter pole was used as the object in this experiment.

5.2.12. Psychometric Curves

To characterize sensory detection thresholds and performance as a function of distance, 5 mice (JF25395, JF25396, JF25400, JF25402, JF25403), after performing on the radial distance task, were trained for 16-18 consecutive days to yield psychometric curves. They had to discriminate 4 different distances (1 mm - 4 mm) which were changed daily. For every distance at least 4 sessions were performed and the distance to discriminate at each daily session was chosen to minimize effects of training history on the performance.

Such effects of training history can impact performance for example when the mouse was trained on 1 mm, a distance the mice often failed to discriminate, the day before.

In sessions with a hardly discriminable distance (1 mm), mice tend to perform randomly (licking at every trial) for the whole session, during which, as they don't practice the task anymore, a decline in proficiency occurs. This can be a temporary effect, but nonetheless impacts the following session. Similar effects can be imagined with easy discriminations as well. Therefore the 2 middle distances (2mm, 3mm) were chosen in an alternating fashion.
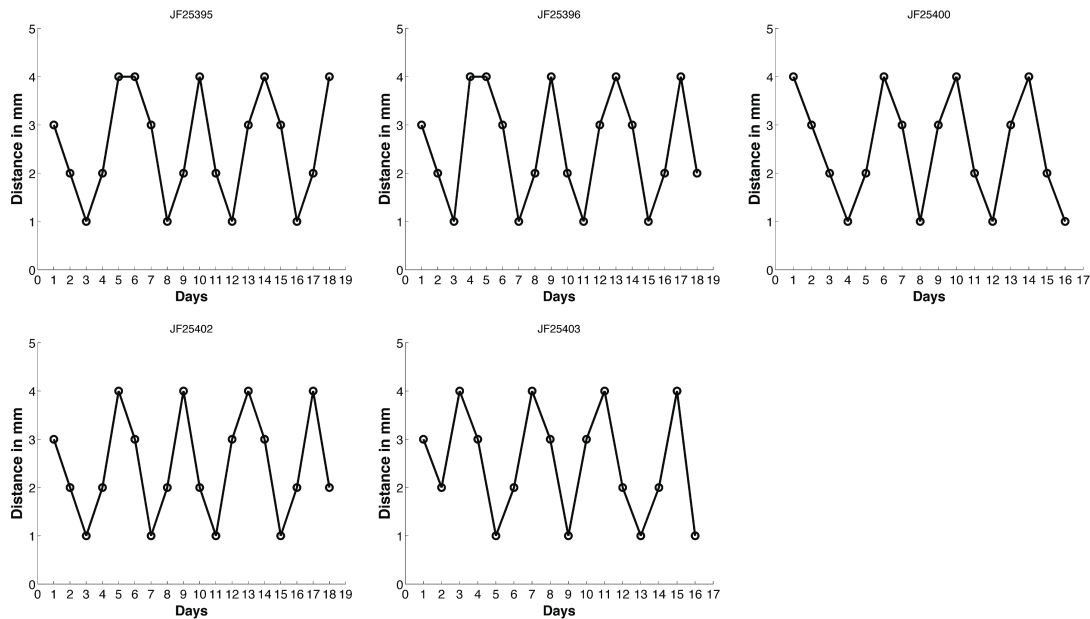

Fig. 5.7: Graphs showing the daily sequence of distances, on which mice were performing during the experiment to acquire psychometric curves. One graph for every mouse, from left to right, top to bottom: JF25395, JF25396, JF25400, JF25402, JF25403

5.2.13. Fooling Experiment

This experiment was conducted to find out whether mice use moment or rate-of-change of moment as a strategy to discriminate radial distance, for which the task had to be altered to some degree. Instead of one go and one no-go position, the participating mice (JF25395, JF25397, JF25399, JF2401, JF25403) had to discriminate between one go position and a range of no-go positions. The range of no-go positions was achieved by defining a proximal border (usually 2 mm distant from the go position) and a distal border (usually 8 mm distant) and then randomly choosing a position between those two borders for each trial (*Fig.5.8*). Over the course of the session (>400 trials) the no-go range was entirely probed which forced the mice to bin the stimuli of the no-go range into one category.
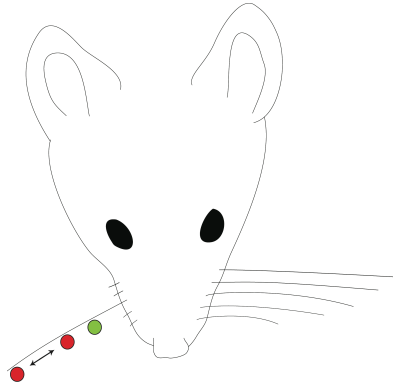
Fig. 5.8: Drawing showing the positions of the go position (green) and the range of no-go positions (red).

After training for ~2 days on the task with a go and a range of no-go positions until high performance levels were reached constantly, the task was again altered to allow one to selectively uncouple object position from the rate-of-change of moment experienced by the mice. This was done by dropping a flexible object into the go-position (***Fig.5.9***) in one arbitrarily chosen trial every 32 trials.

The stiffness of the flexible object was chosen in a way that it would flex when the animal whisks against it, thereby lowering the moment and rate-of-change of moment (RCM) perceived by the mouse. This would allow to have an object in the go position but mimic the moment of the no-go position, thereby creating a somatosensory illusion for the mouse if moment is the dominant cue for radial distance judgement. It also explains why the single no-go position was replaced with a range of no-go positions, as it would be easier to have the moment properties of flexible object contact fall into a range of moments than to exactly match one single no-go moment. The trials in which the flexible object dropped into the whisker field (fooling trials) were neither rewarded nor punished, in order to prevent learning of a third category, but rather to see how the mouse would spontaneously interpret the object, being in the go position but mimicking the rate-of-change of moment of a no-go position. The function[9] used for this experiment put 32 consecutive trials in one block and then chose one fooling trial within this block and one unrewarded and unpunished go trial, again in order to minimize the chance of learning a third stimulus category.
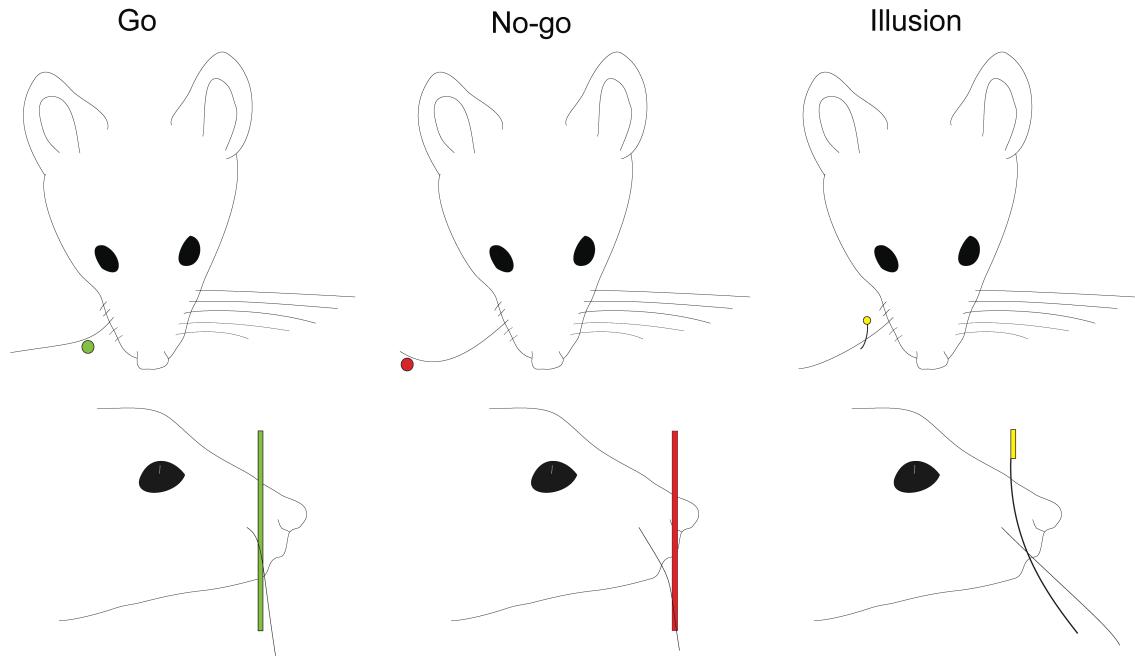
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[9] See Appendix 8.1.3.

Fig. 5.9: Showing the top (upper row) and side (lower row) view of the three trial types in the fooling experiment. From left to right: In the go trials, a stainless steel pole (green) drops close to the face. For the no-go trials, where the stainless steel pole (red) drops more laterally only one particular no-go position is shown for clarity purposes, not the no-go range (***Fig.5.8***). In the illusion trials a flexible object (rat's whisker (black) mounted on metal pole (yellow)) drops into the go position. The flexible object bends when the mouse whisks against it, thereby lowering the moment present in the follicle at contact.

5.2.14. Fooling Experiment Control

In order to see whether the mice perceive the flexible object as a third category and react in whatever way they prefer, a control experiment was conducted with 4 mice (JF25395, JF25397, JF25399, JF25403). This experiment was designed similarly to the fooling experiment but required the use of a different function[10] and instead of the flexible object dropping into the go position, the flexible object dropped once in 32 trials into a position within the no-go range, usually at a distance of 5 mm from the go position. Accordingly there was an unrewarded and unpunished no-go trial once in every 32 trials, instead of the unrewarded and unpunished go trial. An even more flexible object than the one presented in the fooling experiment was used for this experiment, as it was contacted by a more lateral point along the mouse's whisker. Its compliance was chosen to be low enough that the object flexed at contact with the principal whisker, similarly to the flexible object at contact used in the fooling experiment.

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[10] See Appendix 8.1.4.

5.2.15. Control Experiment

To be certain that the mice solve the radial distance task only with somatosensory cues a control experiment was conducted. After losing their principal whisker in the course of the fooling experiment, 3 mice (JF25397, JF25399, JF25401) were continued to be run on the behavioral task without changing the position of the go stimulus. For a total of five days the mice had to discriminate a distance of 5 mm without whiskers. All relevant parameters such as lighting and sounds of motor and pole movement were identical to the 2-position radial distance task.

5.2.16. Data Analysis

Performance Analysis. To extract performance levels from the behavioral data, the first 20 trials and the trials after the mouse stopped performing constantly due to sedation were usually discarded from the session to be analyzed. To calculate the overall performance in the session, the number of correct trials was divided by the total number of trials. To calculate performance in the course of the session, a moving average over 100 sessions was calculated.

Mean Reaction Time Extraction. The mean reaction time, as used for the whisker tracking in the radial distance experiment, was obtained by pooling the timepoints of first beam breaks (licking) of every go trial and then creating a histogram with 20 bins to see the number of first licks at every time point. The maximum value of the histogram was defined as the mean reaction time.

Statistical Analysis. For statistical analysis to test if mouse behavior lies above chance a one sided binomial test was used. The null hypothesis was chance behavior (50% of trials correct). The trials of multiple sessions were pooled. The number of correct trials and the total number of trials were used for the binomial distribution calculation. The resulting p-value is the probability of obtaining the number of correct trials or more if the null hypothesis is true. A significance level of $p<0.05$ is assumed to be statistically significant.

To statistically test wether or not the behavior of the mouse in trial type A is different than the behavior in trial type B a two sided binomial test was applied. The null hypothesis was the performance levels of trial type B. A significance level of $p<0.05$ is assumed to be statistically significant.

In scattering results, significance was assumed if differences were larger than three times the standard deviation.

Analysis of Whisking Behavior. For whisker tracking the custom written whisker tracking software was used (see above). All further analysis and graphs were produced with MATLAB 2007b and MATLAB 2008.

The tracked whisker data, a representation of the whisker's medial axis as a sequence of N points (whisker trace) was further analyzed with MATLAB. For extracting the whisker properties curvature, azimuth (theta) and rostro-caudal position from the movies recorded in the fooling experiment, two regions of interest (ROI) were chosen to be able to compare the data from all three trial types (no-go, go, fooling). The medial border of the first ROI, of which an example is shown (***Fig.5.10***), has been chosen as close as possible to the mouse's face. The lateral border was chosen as close as possible to the point of contact between the whisker and the pole in the go position, which corresponded to ~40 pixels in width. This ROI has been used to extract the curvature of the whisker.
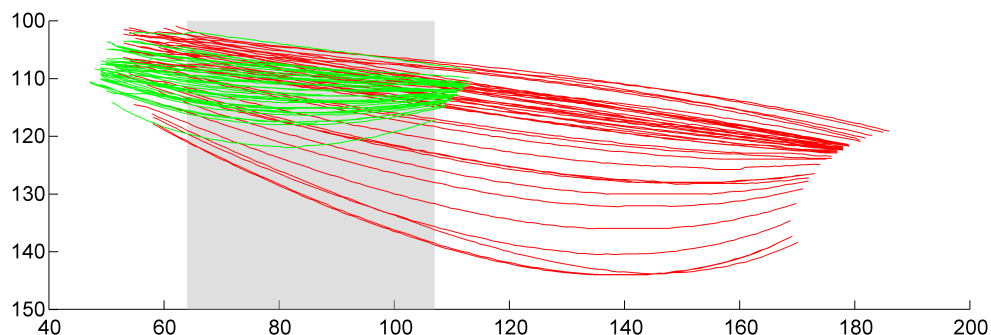

Fig. 5.10: Graphic showing the traces of the tracked whisker in two example trials (JF25399). The traces of the no-go trial in red, go trial in green. In grey the region of interest for curvature extraction. Units are pixels in the tracked movie, y-Axis is anterior-posterior (high values - anterior), x-Axis medial-lateral (high values-lateral).

A second more narrow ROI has been chosen to calculate the azimuth (theta) of the whisker. The medial border of this ROI was the same as for the first ROI, but the lateral border was chosen ~15 pixels from the medial border. The narrow ROI minimizes the effect of whisker curvature on the azimuth values. This is necessary as the azimuth is being calculated by fitting a straight line between the two end points of the whisker splinter within the second ROI and then measuring the angle between the medial border of the ROI and the fitted line. Because of this algorithm the stronger the

curvature the stronger the influence on the slope of the fitted line and therefore also on the calculated angle.

To calculate the mentioned properties azimuth/angle (θ) and curvature, the shape of the whisker was first approximated from the data within the ROI as a parametric curve, c(l) = (x(l),y(l)), where x(l) and y(l) are fifth degree polyomials and l ∈ [0,1]. This was necessary to compute accurate derivatives. The polynomials were computed by fitting xi and yi, respectively, as a function of li, where

$$l_i = \sum_{k=1}^{i} \sqrt{\Delta x_k^2 + \Delta y_k^2} \Big/ \sum_{k=1}^{N} \sqrt{\Delta x_k^2 + \Delta y_k^2}$$

and Δ denotes the backwards difference. Using c(l), angle(θ; in degrees), signed curvature (κ; in 1/mm) and arc length (s; in mm) were computed at each time point as:

$$\theta(t) = \frac{1}{b-a} \int_a^b \tan^{-1}(y'/x') dl \,,$$

$$\kappa(t) = \frac{1}{d-c} \int_c^d \frac{x'y'' - y'x''}{\left(x'^2 + y'^2\right)^{3/2}} dl \,,$$

$$s(l) = \int_0^l \sqrt{x'^2 + y'^2} dl \,,$$

where the intervals [*a, b*] and [*c, d*] denote regions of interest over which to average. Note that these intervals are defined in terms of *s(l)*. *θ* gives the azimuthal angle computed with respect to the midline. Protraction corresponds to increasing angle values. *θ* = 0 is perpendicular to the midline of the mouse.

The last parameter extracted from the whisker traces was the rostro-caudal position of the whisker. It is defined as the y-value of the pixel at the intersection point between the medial border of the ROI and the curve fitted to the whisker for the curvature extraction. In some cases, the values of rostro-caudal position have been transformed to millimeter values. These millimeter values have been calculated from pixel values, the correlations for which were empirically approximated. To do so, two mice (JF25403 and JF25395) where anesthetized and two dots were painted on the whisker shaft. The first one placed around 5 mm lateral from the follicle, the second one around 10 mm from

the follicle, both distances have been measured with a micro-ruler. Then the mice were placed in the behavioral apparatus and movies were recorded while performing in the task. For every mouse, the number of pixels between the two points were measured in 10 frames, each frame of a different movie, and correlated with the known millimeter values (JF25395: 1mm = 12.97 pixels, std = 0.036mm. JF25403: 1mm = 14.83 pixels, std = 0.076mm). For JF25395 and JF25403 these calculated values were used when transforming pixel in mm values, for all other mice the mean value (1mm = 13.90 pixels) was used.

6. Results

The first experiment was conducted to find out which strategy mice prefer to use for solving a radial distance discrimination task with multiple whiskers. Mice prefer to use a binary strategy - if the short whisker B2 contacts the stimulus, it must be a go, otherwise a no-go. Therefore, they use a labeled-line strategy to discriminate stimuli differing in their position along the medio-lateral axis in a head fixed behavioral setup. The second experiment shows that mice can make radial distance discriminations with a single whisker, which contacts both go and no-go positions. Mice most likely do not use azimuthal cues for this discrimination. The last experiment was designed to answer whether or not mice use moment or rate-of-change of moment when doing pure radial object distance judgements and gives evidence that mice don't use moment cues to do so.

6.1. Strategies for Radial Distance Discrimination with Multiple Whiskers

The whiskers of the mouse are not all the same: more rostral whiskers are shorter. This arrangement supports a simple strategy to judge radial object distance. By monitoring which of the whisker arcs hit an object, and depending on the contact pattern, the mouse could extract an estimate of distance. Alternatively, radial object location could be inferred by monitoring moment or rate-of-change of moment (RCM) differences, axial forces or azimuthal cues.

Based on an aperture discrimination task, previous studies have argued that single whiskers are not sufficient for radial distance discrimination and that integration over multiple whiskers is required (Krupa et al., 2001).

In this first experiment, mice were free to choose from different strategies to discriminate between two objects differing in their medio-lateral position (3 mm offset). In particular two whiskers (B2 and D2) were trimmed to a length so they could contact only the go position (*Fig.6.1* top). The mice could choose to only monitor B2 or D2 to solve the task: if B2 or D2 contacts the pole then it must be in the go position, otherwise in the no-go position.
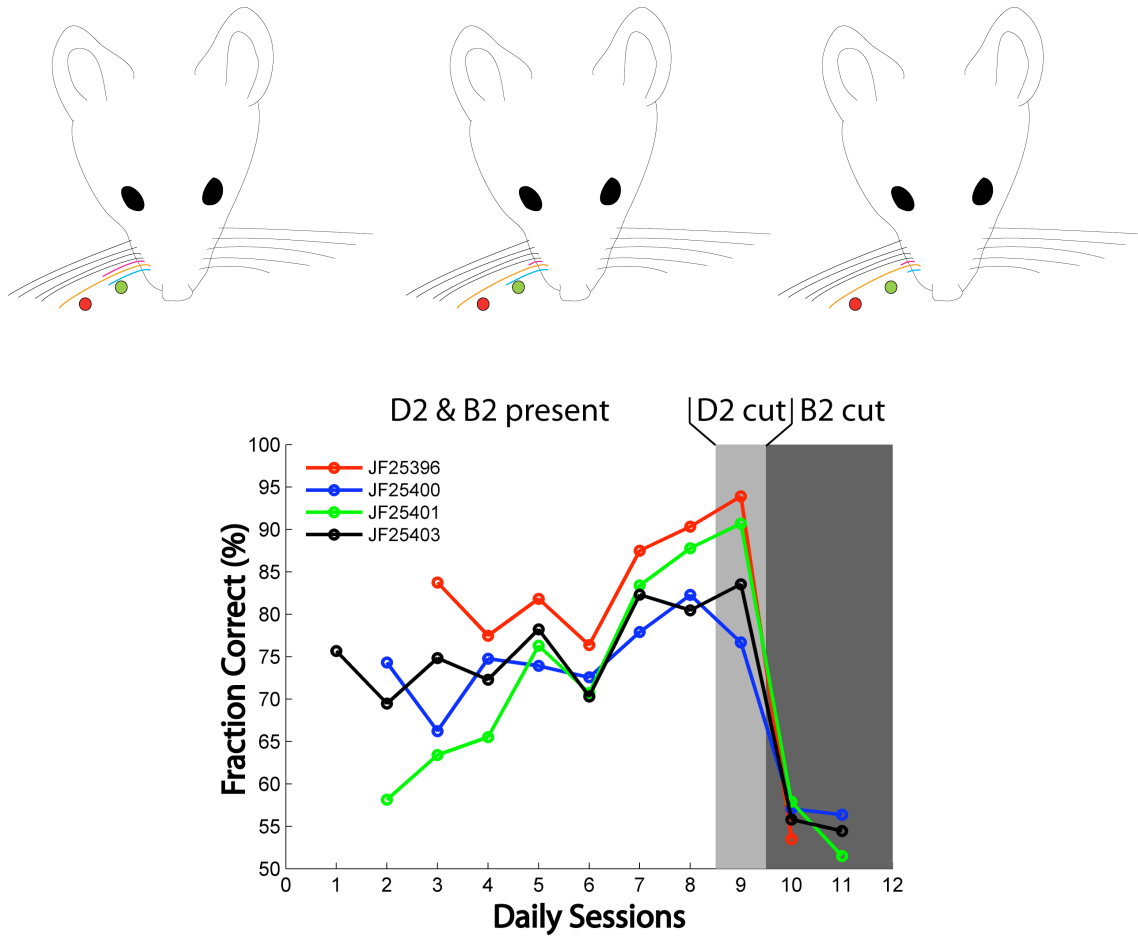
Fig. 6.1: Top figure showing the three trimming statuses of the whisker field during the experiment. From right to left, B2 (pink) and D2 (blue) short (first trimmed state), D2 trimmed and last B2 and D2 trimmed. ~7 posterior whiskers (black) were present throughout the experiment.

Bottom graph shows the performance of the mice throughout the experiment. First point of the performance curves corresponds to the first day of performance after the whisker field was trimmed from full whisker field to the first trimmed state. Light-grey denotes performance on day after trimming of D2, dark-grey marks the two days of performance without D2 or B2.

(**Fig.6.1**) summarizes the performance of the five mice for every session. From the data one can conclude that the mice adapted to their trimmed whisker field and learned to report the position of the stimulus with high accuracy after about a week of daily training, as can be seen in the gradual performance increase until day 8. Linear regression for the data from day 2 - day 8 predicts a mean performance of 80% for the day of D2 trimming (day 9). The mean performance of day 9 is 82% and the mean standard deviation for of days 2-10 is 4.9. The difference of the predicted mean and the actual mean is ~40% of the std. Therefore trimming of D2 has no significant effect on the performance of the mice (significance is assumed when the difference exceeds the std by a factor of 3) but rather mice show a slight increase in performance similar to that on the previous days. Linear regression for the data from day 2 - day 9 predicts

83% performance for the day of trimming B2 (day 10). The actual mean performance on day 10 is 55%. The difference of the predicted mean and the actual mean is 5.7 times larger than the mean standard deviation. Therefore trimming of B2 leads to a significant decrease of the performance of the mice. In a head fixed radial distance discrimination task not every whisker was equally important as trimming D2 and B2 had fundamentally different impacts on performance - trimming of D2 had no significant effect on performance, B2 trimming on the other hand lead to performance levels slightly but significantly above chance (mean performance = 55%, one sided binomial test, $p < 0.0001$). This decrease persisted for several days in all tested mice. The data therefore suggests that the mice choose to adopt a labelled line strategy to solve the task.

6.2. Radial Distance Experiment

We next asked if mice can do pure radial distance discrimination. Mice were able to contact go and no-go position only with one whisker. The positions were adjusted so that the whisker contacted both positions with the same azimuth. The goal was to have mice performing on a 3 mm discrimination task for at least 100 trials with >90% performance and then to quantify azimuthal jitter at contact to see whether azimuthal cues are strong enough to be used to solve the task. As potential azimuthal cues one can imagine if the go and no-go positions are not chosen perfectly and a significant difference remains between whisker azimuth at contact with go versus azimuth at contact with the no-go position. As mice contact the pole multiple times (typically 3-6 contacts before the decision) in every trial and the azimuth of the whisker at contact is not always the same at every contact due to translation, another cue for differentiating go and no-go positions could be the distribution of the azimuths over multiple contacts (greater spread for objects closer to the follicle) (***Fig.6.2***).

Of all 8 mice trained on the radial distance task every mouse except JF25400 was able to reach performance levels above 90% for 100 consecutive trials at 3 mm distance discrimination in the course of this experiment.
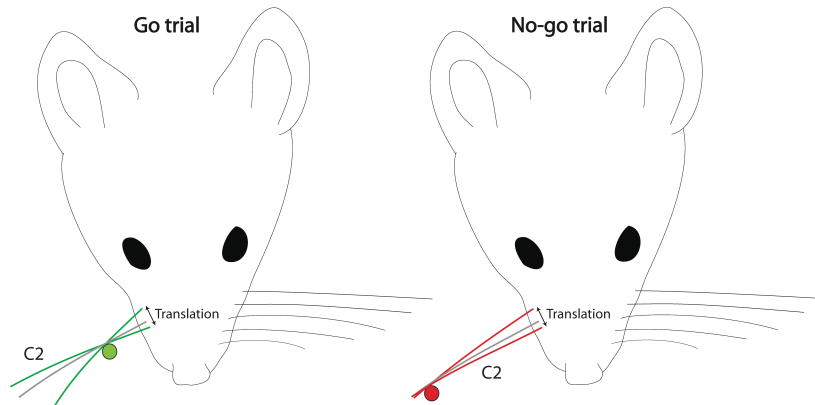
Fig. 6.2: Schematic drawing showing the difference in azimuthal (angular) spread at a given translational offset of one whisker (C2) when contacting a proximal object (left drawing, go trial) or a distal object (right drawing, no-go trial). 3 whisker positions differing in their translational position are shown: extreme positions colored (green - go, red - no-go) and the position contacting go and no-go at the same azimuth (grey). Comparing the two drawings, it can be seen that the difference in azimuth of the two colored whiskers is greater for contacting the proximal object than when contacting the distal object, even though the follicles are at the same position along the whisker pad (translation) and the positions of the objects are well chosen as at one whisker position (grey whisker) the azimuth is the same for both positions.
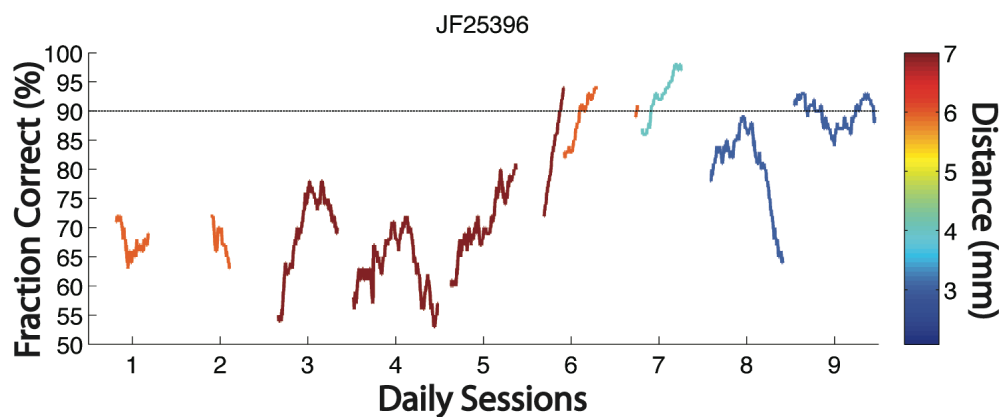

Fig. 6.3: Graph shows the learning curve (moving average over 100 trials) of mouse JF25396 as an example for radial distance learning. Day one is defined as the first day after trimming the whiskerfield to only allow C2 contact. Colors of the curves show the distance in mm the mice had to discriminate in the respective session. Color-distance relationship given by the color bar at the right of the graph.

Of the 7 mice, 3 mice (JF25397, JF25402 and JF25403) were selected and for those 3 mice one 3 mm distance discrimination session was chosen for further analysis of whisker properties at contact. The selection criteria were whether the mouse crossed 90% performance levels and if the mouse seemed to be whisking calmly. Of these three sessions (one per mouse), a sequence of 100 trials was selected with the same criteria as for the session selection and within these 100 trials the C2 whisker of the mouse was tracked in a total of 40 trials, 20 randomly chosen go trials and 20 randomly chosen no-go trials (***Fig.6.4***).
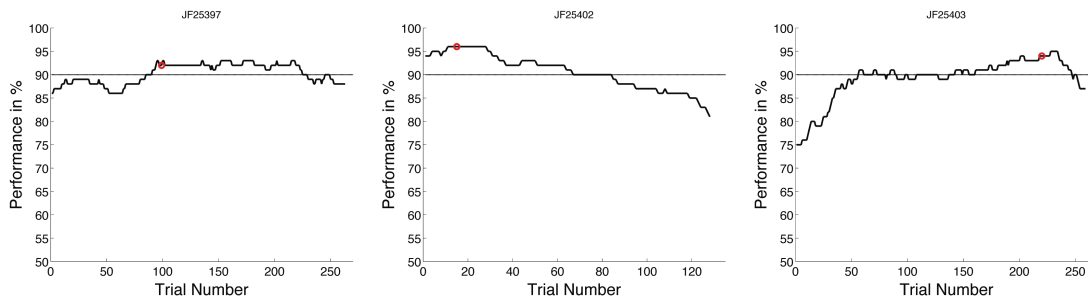
Fig. 6.4: Graphs showing the sessions selected for whisker tracking and further analysis. From left to right the performance (running average over 100 trials) of JF25397, JF25402, JF25403 in the selected sessions. The straight line indicates the threshold of 90% performance level, the red circle indicates the performance level of the 100 trials selected for analysis (JF25397 = 92%, JF25402 = 96%, JF25403 = 94%). The x-value of the red circle is the trial number of the first trial in the sequence of 100 selected trials for tracking.

All three selected mice were performing with high accuracy (JF25397 = 92%, JF25402 = 96%, JF25403 = 94%) and whisked calmly contacting the pole via a posterior to anterior motion.

In order to answer questions about whether the mice solve the task in a purely radial way it is important to have exact quantitative information about properties of whisking. Of the randomly chosen trials not the whole 1.5 seconds of the movie were tracked but a mean reaction time for every session was calculated (for description see methods chapter). All no-go trials were tracked from the beginning until the mean reaction time (mean timepoint of first lick after decision in the go trials). The go trials were also tracked until the mean reaction time, unless the first lick of the to be tracked trial occurred after the mean reaction time. If this was the case the particular go trial was tracked until the time point of the first beam break (lick).

Tracking was started one frame prior to initial contact (the timepoint at which first contact between whisker and pole is established) and ended with the first frame after losing contact. That way one knows the correct frame of initial contact for every contact within the tracking window of the movie.

As a first analysis using the data of the tracked whisker (C2), it was checked whether go and no-go positions were chosen correctly by creating box plots of the azimuth (theta) of the whisker at all initial contacts of every tracked trial for every mouse (**Fig. 6.5**).
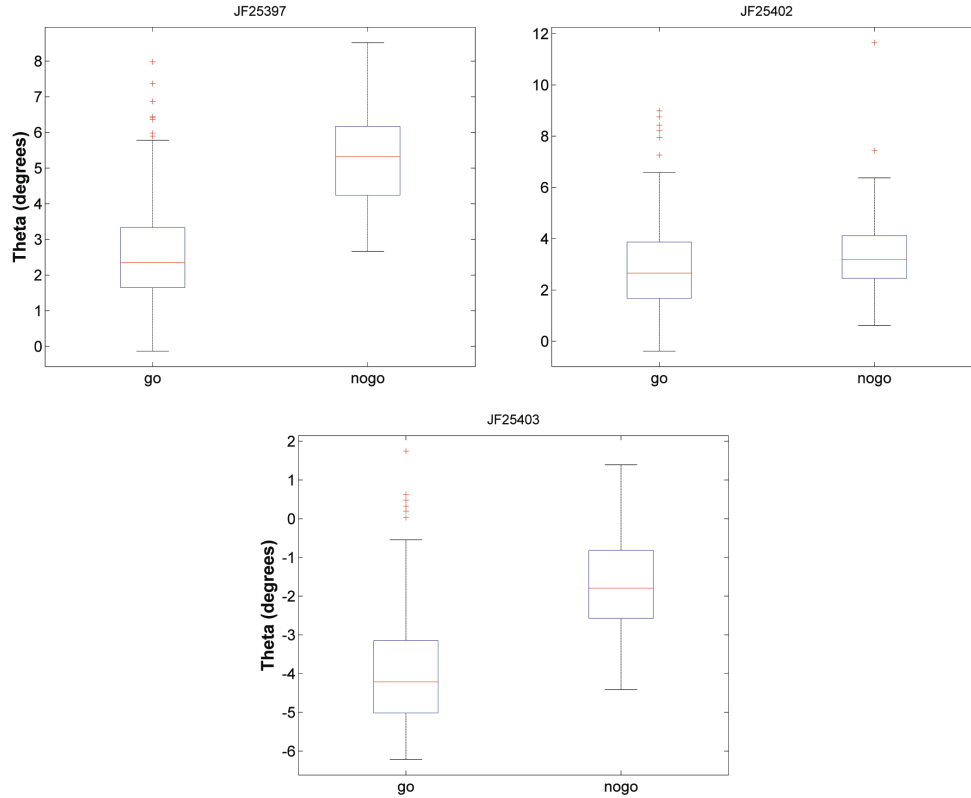
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Fig. 6.5: Graphs show boxplots comparing theta (azimuth) of the principal whisker at initial contact with the pole in the two trial categories (go and no-go) for three mice JF25397 (go: n = 107, no-go: n = 89), JF25402 (go: n = 209, no-go: n = 130), JF25403 (go: n = 122, no-go: n = 100). The red line indicates the median value, the blue box the lower quartile, black whiskers extend to most extreme values within 1.5 times the interquartile range, outliers are defined as values beyond whiskers and indicated with red crosses. Accuracy of position definition can be read off the graphs, where the same median theta value for go and no-go trials would indicate perfect positioning.

This representation of the data (***Fig.6.5***) lets one control whether the go and no-go positions were chosen accurately so that the whisker has the same azimuth at contact with both positions. This is necessary to rule out that azimuthal differences between go and no-go were available to the mice for solving the task. By comparing the mean azimuth of initial contact between go and no-go trials, one sees that the angular differences between the two trials are significant (two sample t-test, $p = 0.006$) but negligible for JF25402 (difference of the mean = 0.5°, standard deviation (std) for go = 1.6, std no-go = 1.3), and significant (two sample t-test, $p < 0.0001$) but small for JF25397 (mean difference = 2.5°, std go = 1.7, std no-go = 1.4) and JF25403 (mean difference = 2.1°, std go = 1.6, std no-go = 1.2). From azimuthal psychometric curves (O'Connor et al., submitted) it is known that animals can detect differences in azimuthal angle of 5.6° above chance (fraction correct = 60%-70%). These psychometric curves were obtained with mice that had a whisker field of more than one whisker (usually 4)

to contact the pole. In the cited study two mice were also trained on 2.8° azimuthal difference, at which they performed with accuracy levels of about 53% and therefore only marginally above chance (one-sided binomial test, p=0.0625). Regarding the fact that single whisker object localization is harder than with a multi-whisker whisker field (O'Connor et al., submitted) and the smaller angular differences between go and no-go, but higher performance levels in the radial distance experiment compared to the cited study, one can conclude that azimuthal differences between go and no-go were not the cue used by the mice to discriminate go from no-go.

As whisking has two components, change of azimuth and change of translation, and those two are to some extent independent from each other, another possibility to solve the task would be for the mouse to contact the object multiple times within one trial and altering one component over the other (***Fig.6.2***). To analyze this relationship a 2D-analysis of the two components at every initial contact has been conducted (***Fig.6.6***).
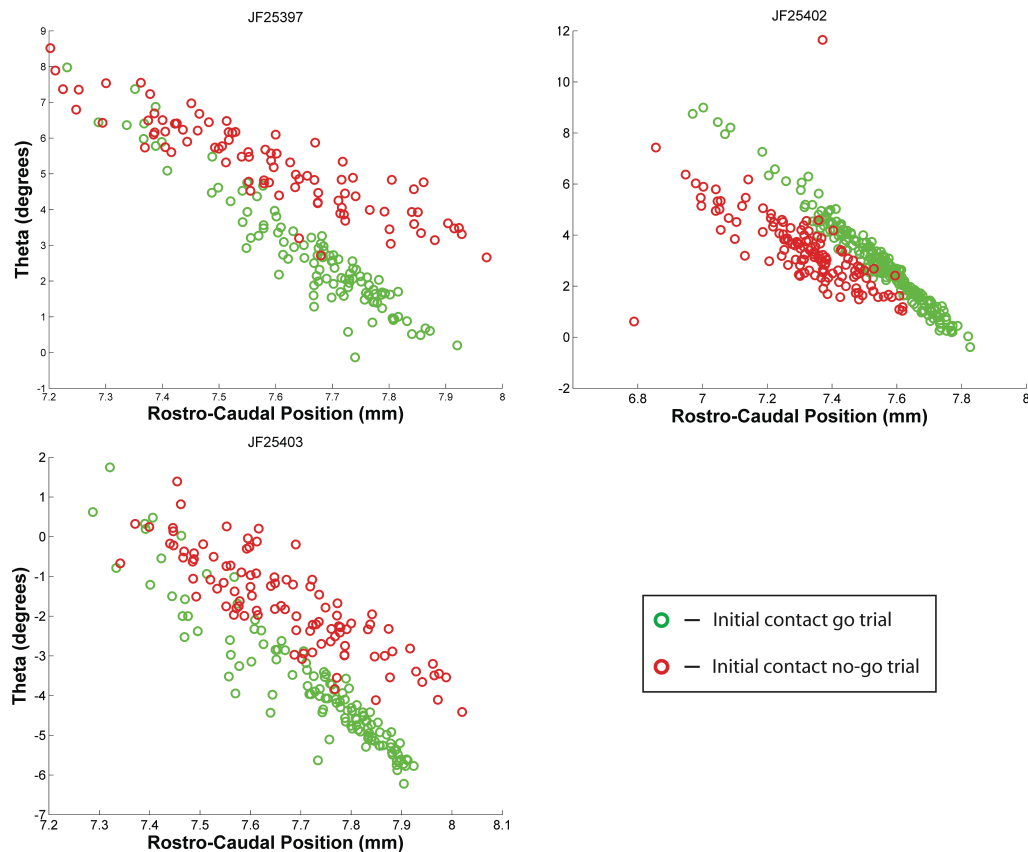
Fig. 6.6: Plots showing the correlation of rostro-caudal position and azimuth (theta) of the whisker at initial contact with the go stimulus (green circles) and no-go stimulus (red circles) for three mice. Noticeable is the separation of the two data point clusters, as well as the difference in slope between the clusters of each category, the latter of which could only be extracted by monitoring both variables and integrating information over multiple contacts.

Initial contacts of go (green circles) and no-go (red circles) plotted according to their azimuthal and translational values are separated in clusters (*Fig.6.6*). The cluster separation is the 2 dimensional visualization of the not quite perfect definition of go and no-go positions already seen in the median azimuth difference in the box plots. Accordingly the separation is most likely below the azimuthal discrimination thresholds (O'Connor et al., submitted).

A true difference between go and no-go position however could be extracted by contacting the object multiple times within one trial and altering one whisking component over the other (azimuth and translation). When integrating over multiple initial contacts within one trial and monitoring azimuth and translation this behavior would yield different information (different azimuthal spread) for each stimulus category. This information is visualized in form of the slope of each cluster (*Fig.6.6*). The slope of the go trials is always more negative than the one of the no-go trials, which corresponds to a greater azimuthal change at a given translational change for the go trials (schematic depiction in *Fig.6.2*).

In order to extract this information reliably the mice would have to probe as much of the space along the long axis of the clusters as possible. In fact looking at theta of multiple contacts within one trial one can see in some trials considerable (>6°) azimuthal spread within single trials (*Fig.6.7*). In the go trials the azimuth of the whisker at initial contact becomes gradually smaller during the course of multiple contacts within one trial. This means that the mouse first contacts the object with the follicle relatively far posterior and from one contact to the next gradually shifts the follicle more anterior, thereby changing the azimuth to a more retracted one. In the no-go trials on the other hand there is no clear shift but more random and much smaller differences in azimuth over multiple contacts within single trials (*Fig.6.7*).

Another way of representing this data is to plot the whisker traces of each initial contact into a movie frame for every trial. This data is only shown for mouse JF25402 (*Fig.6.8*).
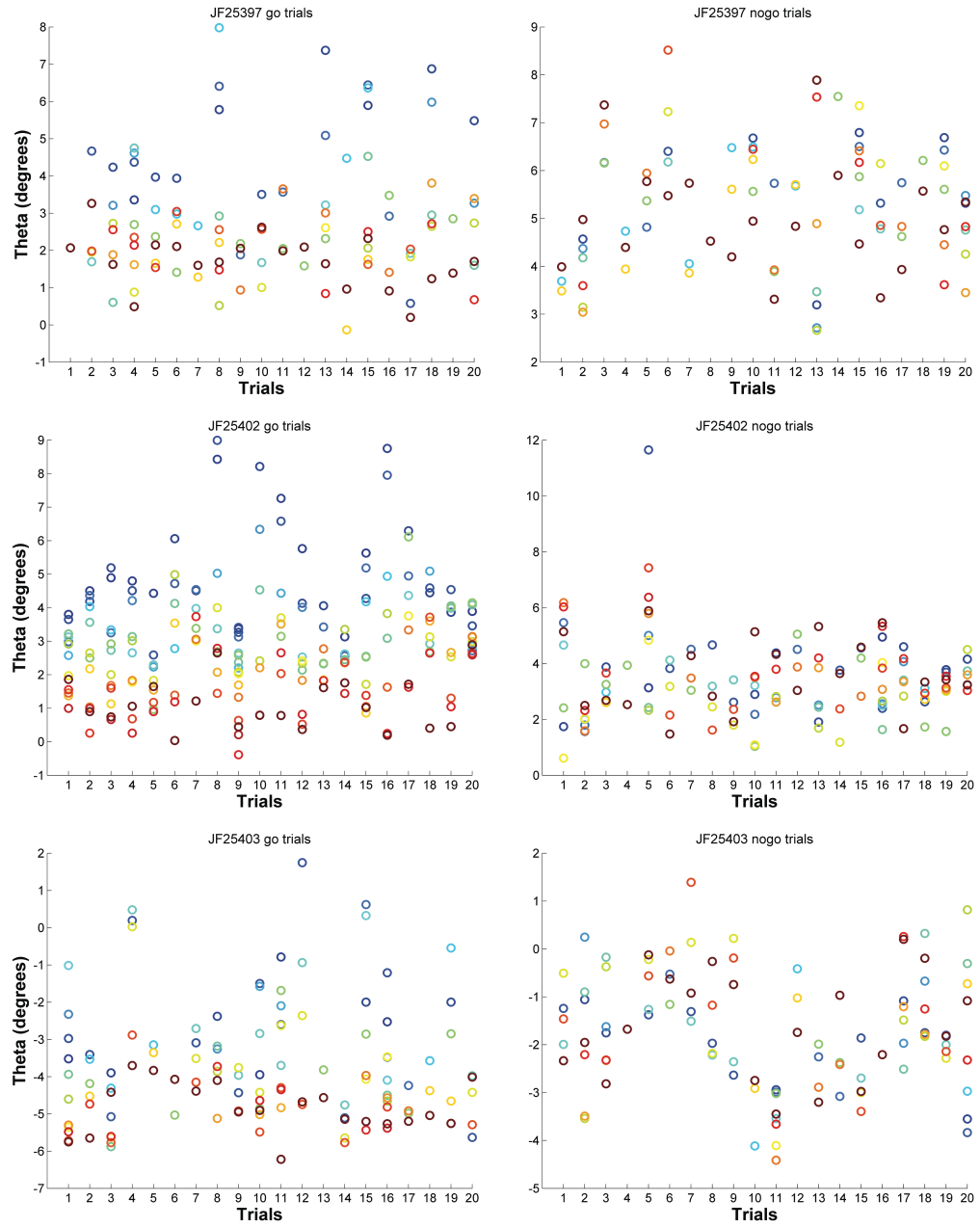
Fig. 6.7: Graphs showing the azimuthal spread within single go trials (left column) and no-go trials (right column) for mice JF25397 (top row), JF25402 (middle row), JF25403 (bottom row). Each data point shows the azimuth of the whisker at one initial contact, the color of the point marks the temporal sequence of the contacts within one trial (blue being early in the trial, red being late). Contacts in go trials show greater spread of and temporal shift of azimuth, no-go trials show smaller spreads and don't seem to have a temporal trend.
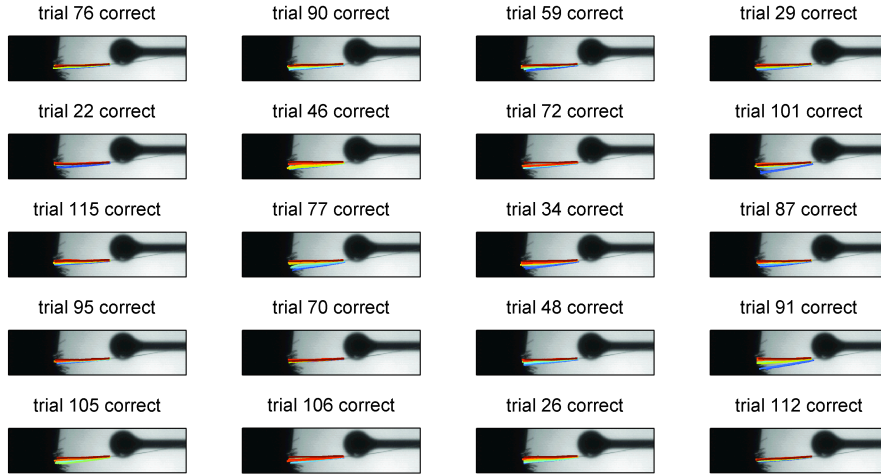
Fig. 6.8: Whisker traces of all tracked trials (go trials first five rows, no-go trials below) of mouse JF25402 plotted into two sections of movie frames (one from a go trial, the other one from a no-go trial). In both frames the head of the mouse is on the left side, anterior is up, posterior is down. Every trial is marked with its number and whether or not the response of the mouse was correct or not (all were 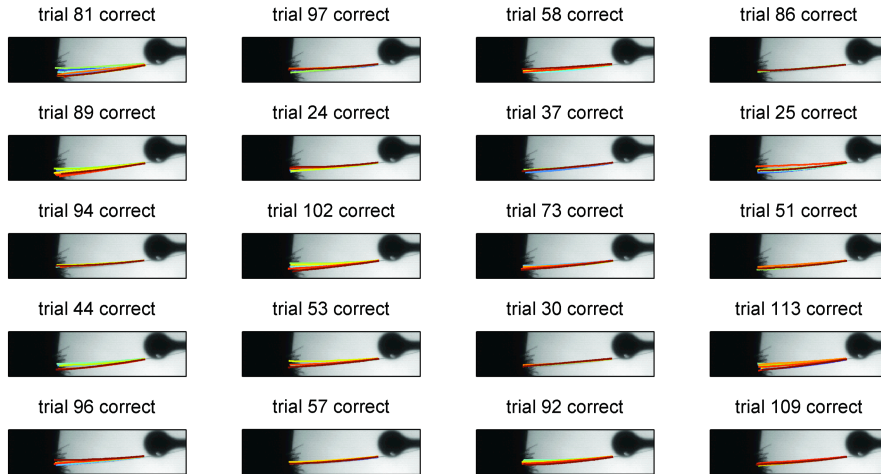correct). Color of whisker traces marks the temporal sequence of individual contacts within one trial (blue being early in the trial, red being late). Visible in the color of whisker traces in the go trials is the shift of azimuth over multiple contacts.

The data of whisker azimuth over multiple contacts within each trial (**Fig.6.7**, **Fig.6.8**) shows that there are trials with significant differences in whisker position over initial contacts, but there are also trials with very little spread. In both cases the mouse reports the position correctly.

To make quantitative arguments a histogram of the azimuthal spread of each trial has been created (**Fig.6.9**). The azimuthal spread is defined as the difference of the two most extreme angles of the whisker at initial contacts within one trial. For every trial the

azimuthal spread was calculated and constituted one data point. Then a histogram has been produced from these data points showing the number of trials with a given azimuthal spread. From this the percentage of trials was calculated, where the azimuthal spread surpasses the detection limit of about 4° (O'Connor et al., submitted).
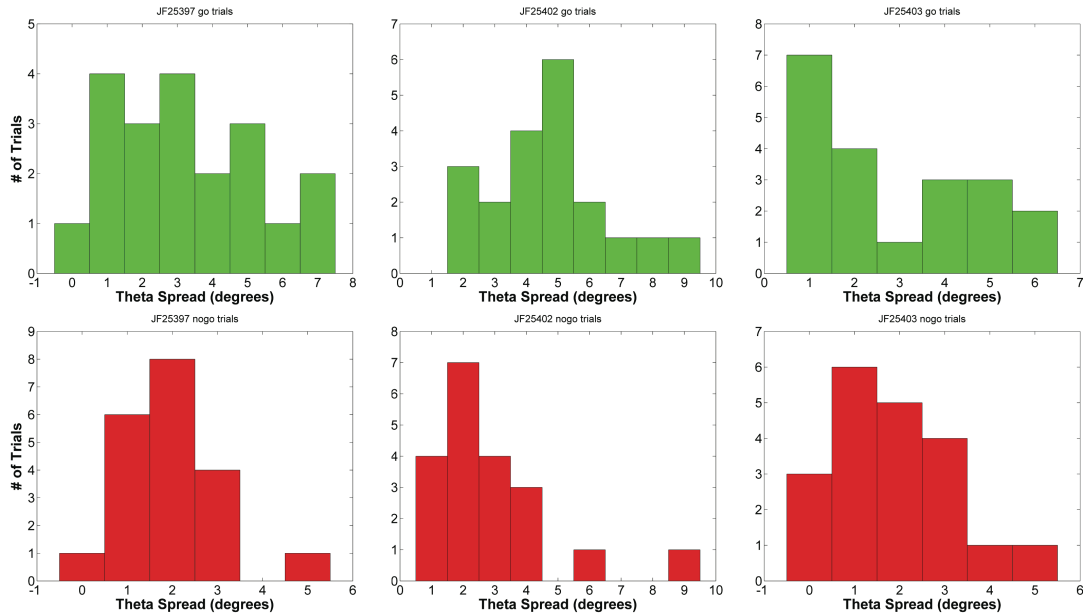

Fig. 6.9: Histograms showing the number of trials for every degree of azimuthal (theta) spread of the whisker over multiple contacts within one trial. Top row showing go trials (green), bottom row showing no-go trials (red), columns from left to right: JF25397, JF25402, JF25403.

The histograms (**Fig.6.9**) show that - except for the go trials of mouse JF25402 - nowhere does the spread of whisker azimuth at initial contact surpass the azimuthal detection limit (4°) in a majority of trials. The spread of whisker azimuth is below 4° in 87% of the no-go trials across animals (JF25397 - 95%, JF25402 - 75%, JF25403 - 90%) and below 4° in 48% of the go trials across animals (JF25397 - 69%, JF25402 - 25%, JF25403 - 60%). Even though large fractions of trials are below the detection threshold, the mice solve the task with an accuracy of 92-96%.

As this spread is the only possibility for the mice to solve the task with azimuthal cues, we can conclude that in fact mice can discriminate radial distances via non azimuth dependent strategies.

Summed up one can say, that azimuthal cues for radial distance discrimination exist even in single whisker behavior. The first azimuthal cue is owed to the fact that it is hard to define go and no-go positions with an accuracy high enough, that the mean

azimuthal difference at first contact is below 2°. The second azimuthal cue arises from trigonometric rules, which lead to a situation where by integrating azimuthal and translational information over multiple contacts within one trial and independently altering the translational and azimuthal values from contact to contact, a distinctive relationship between change of translation and change of azimuth holds information to distinguish the two radial positions. However, due to the small angular differences between the two positions and the small angular spread within multiple contacts of most trials, both of which are below angular values reliably distinguishable for mice, it is unlikely that these cues are used for discrimination. Rather mice can reliably distinguish radial distances without the use of azimuthal cues, but with a purely radial distance discrimination strategy.

6.3. Psychometric Curves

In order to know sensory detection thresholds of radial distance perception psychometric curve experiments have been conducted with 5 mice (JF25395, JF25396, JF25400, JF25402, JF25403). As described in the methods section above, the mice had to discriminate four distances: 1 mm, 2 mm, 3 mm and 4 mm, each distance in at least four daily sessions. The positions of go and no-go were defined in a way to minimize azimuthal differences of the whisker when contacting the two positions.

The sequence of distances on which the mice were trained was chosen to minimize effects of training history on overall performance levels (for description see methods chapter).

To compare performance levels at discriminating different distances the behavioral performance was analyzed (*Fig.6.10*). In order to show that the mice were already expert performers when starting the experiment and no major learning of the task was going on during the experiments the color code of the data points indicates the training day (*Fig.6.10*). Except for mouse JF25400 which started off at low performance and showed improvement during the experiment, the data can be seen as the best single whisker radial distance performance these 4 mice were able to do.
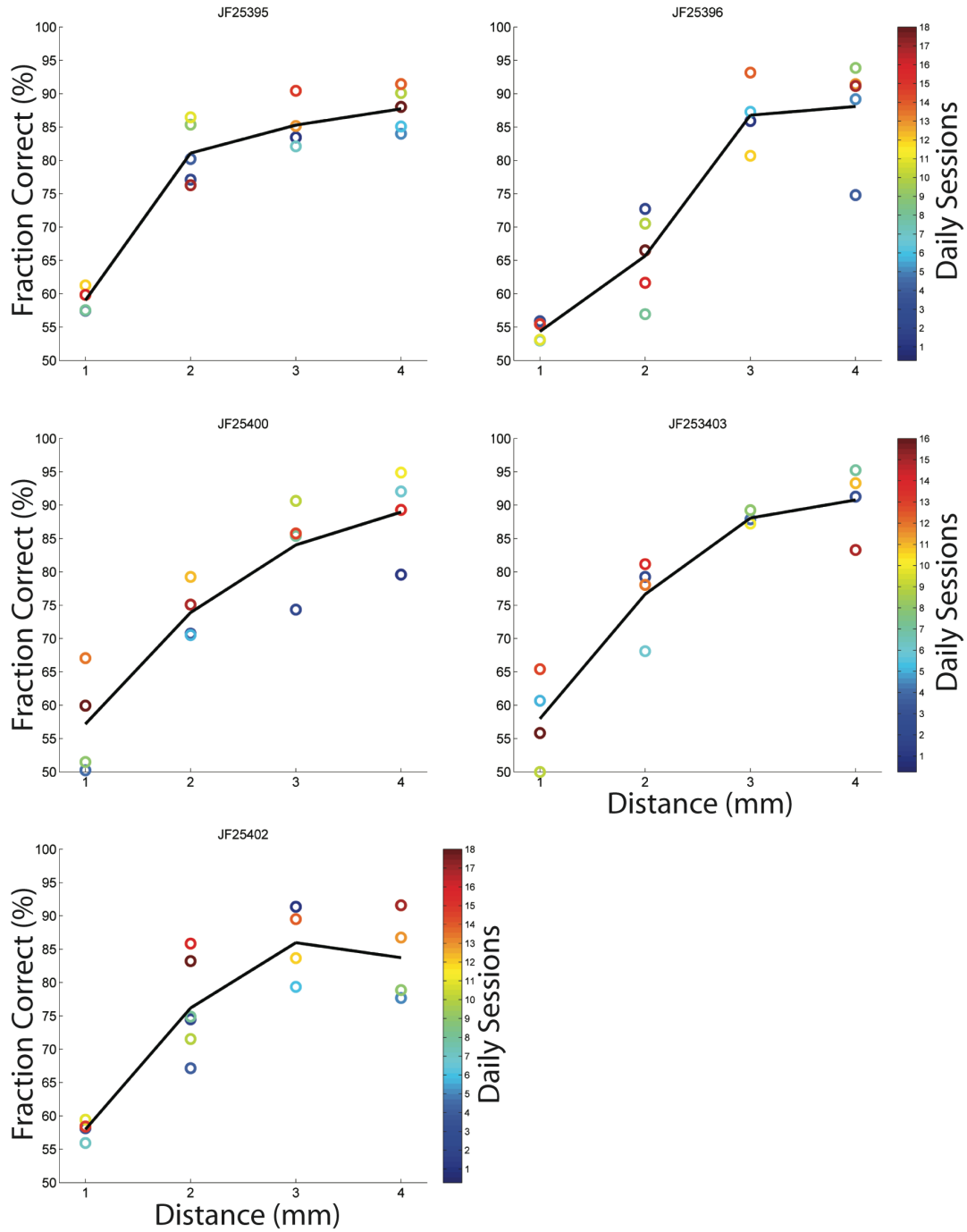
Fig. 6.10: Psychometric curves showing the performance of 5 mice at 4 different radial distance discriminations with a single whisker. Black line indicates the mean performance at each distance, colored circles the performance levels in individual sessions. Color of the circles indicate day of the experiment, readable from the color bar at the right of each row.

The performance differed between the individual mice most profoundly in their interday variability of accuracy. Mouse JF25395 for example has very constant performance levels within a range of 10%, JF25402 and JF25396 on the other hand had large deviations in their performance depending on their daily situation. Generally mice seem

to have similar detection thresholds and their performances also seem to asymptote at roughly similar distances.

We can say that the detection threshold of this batch of mice in discriminating radial distances with a single whisker lies slightly below 1 mm, as the performance when discriminating 1 mm is slightly but significantly (one sided binomial test) above chance levels (JF25395 = 59%, JF25396 = 54%, JF25400 = 57%, JF25402 = 58%, JF25403 = 58%). Furthermore the mean performance increase between 3 mm and 4 mm is small (JF25395 = +2.45%, JF25396 = +1.31%, JF25400 = +4.92%, JF25402 = -2.25%, JF25403 = +2.71%), which shows that distances of more than 3 mm become distinguishable without much ambiguity for the mice. Also the observation made in many training sessions that overall session performance of mice hardly exceeds 90-95% and the 4 mm performances (JF25395 = 88%, JF25396 = 88% , JF25400 = 89%, JF25402 = 84%, JF25403 = 91%) come close to that value, increasing the distance will most probably not have a big effect on the performance levels, as the asymptoting phase of the curve is reached at 4 mm distances.

6.4. Fooling Experiment

After arguing that mice do not use azimuthal cues to judge radial distance, the question remains which cues are in fact used. It is of great interest to know the exact mechanical variable used to judge object location in the medio-lateral axis, when doing systems neurobiological research in the somatosensory system. Multiple strategies can be imagined to be used by mice to differentiate between tactile stimuli along their whiskers. Backed by extensive mechanical characterizations of rats whiskers, it was proposed that moment or rate-of-change of moment (RCM) can be used as a prominent mechanical cue to discriminate radial distance (Birdwell et al., 2007). As whiskers are tapered objects, the force at contact becomes smaller the more distally the stimulus is located. Also the fact that "touch cells" in the trigeminal ganglion show increased firing rates when the distance between object and whisker base decreases, supported this view (Szwed et al., 2006).

To empirically test this hypothesis, an experiment was designed which allowed altering moment and RCM independently from object location. To do so, a flexible object was dropped into the go position in a subset of trials (fooling trials), the object was chosen to have an only slightly higher compliance than the principal whisker of the mice. At

contact the object bent, thereby reducing the perceived moment and mimicking the moment cues of the no-go stimulus, while actually being located in the go position. Therefore, if the mice rely on moment or RCM cues for solving the task, the flexible object would create a somatosensory illusion and they would not lick in the fooling trials. As it would be hard to exactly match the moment of a single no-go position, a range of no-go positions (typically 2 mm - 8 mm from the go position) was used instead. A total of 5 mice (JF25395, JF25397, JF25399, JF25401 and JF25403) were behaving in the task.

To confirm that the compliance of the flexible object used fell into the right range, the principal whisker of 2 mice (JF25395 and JF25399) was tracked in all fooling trials of the experiments. The whisker was tracked throughout the whole 1.5 seconds of the movie. For mouse JF25395, 20 randomly chosen go trials and 24 randomly chosen no-go trials (4 trials of every mm of the no-go range) respectively from two sessions (day 1 and day 4 of 4 days) were tracked. For mouse JF25399 in two sessions (day 5 and day 6 of 6 days) 36 randomly chosen go trials and 36 randomly chosen no-go trials (6 trials for every mm of the no-go range) were tracked.

A visual readout of the force present in the follicle is the curvature of the whisker which can be extracted from the tracked movies (for description see methods chapter). The curvature was then plotted against the rostro-caudal position of the whisker to compare the force upon contact at a defined protraction state in the different trials (go, no-go and fooling). The whisker trace of each tracked frame was one data point. For analysis, the data points of multiple days were binned according to the category of the stimulus. The eight categories were: go trial, position within the no-go range (6 categories) and illusion trial. In which of the 6 no-go categories a data point fell depended on the position of the pole within the no-go range in that particular trial. The no-go range was divided in 6 parts, each comprising 1 mm of the no-go range, differing in their distance from the medial border (6 bins defined by distance from the medial border of the no-go range: 0-1mm, 1-2mm, 2-3mm, 3-4mm, 4-5mm, 5-6mm).

Within those 8 categories the whisker traces were further binned according to the rostro-caudal position of the whisker. The minimum and maximum value of rostro-caudal position of all whisker traces of each category were taken to define the extreme points of whisking for every category. The distance between those two points was divided in 20 bins and each data point was then placed into one of those bins

according to its rostro-caudal position value. That way, multiple data points for each position of the whisker within each category could be averaged and analyzed.

When plotting whisker position versus curvature, the more proximal the object, the higher the curvature of the whisker for a given protraction state, therefore higher values for go trials. The curvature of the whisker in the fooling trials should ideally fall within the range of the no-go curves. These curves are shown for JF25399 and JF25395, where all tracked illusion trials and all tracked go trials were pooled (**Fig.6.11**). The tracked no-go trials were plotted depending on their position within the no-go range in 1 mm bins. No-go data has errorbars of similar size as the go data, but are not shown for the no-go curves, as one would not be able to make out the curves anymore.
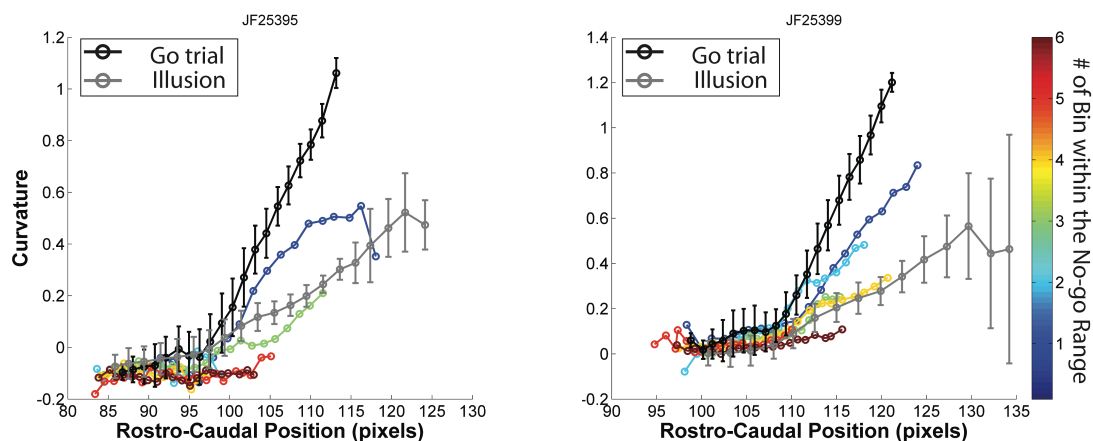

Fig. 6.11: Whisker position dependent curvature curves for JF25395 (left graph), JF25399 (right graph) comparing curvature in go trials (black), range of no-go trials (colored) and in fooling trials with the flexible object (grey). The position of the no-go stimuli within the no-go range can be read from the color of the curve and the colorbar. The colorbar indicates color and the corresponding number of the bin in the no-go range. Distances from the medial border of the no-go range: bin1: 0-1mm, bin2: 1-2mm,...,bin6: 5-6mm. Errorbars are given for the curvature of the whisker in the go trials and fooling trials, but are not shown for the no-go trials, for visual clarity purposes.

The graphs show the curves of the go and no-go trials roughly as expected (**Fig.6.11**). The part of the curve between 82 to 98 for JF25395 and 95 to 110 for JF25399 do show to some extent the same order as the values of stronger protraction as is expected by the linear deflection properties of the whisker but in the data of mouse JF25395 there are irregularities in this initial phase of the curves. The curve of the fooling stimulus lies in parts above the go curve, so do some of the curves of the no-go categories. This can be explained by the resolution of curvature measurement which is not high enough to accurately extract small curvature differences. Additionally, as the frame prior to contact was tracked as well and not discarded in order to have baseline

curvature values, some part of this initial phase of the curves are curvature values prior to contact. The curvature differences between the different categories and the curvature increase in this region of the curves is smaller than the standard deviation of the single data points. This initial region therefore holds little significant information and all following interpretations are based on the values in the second part (rising phase) of the curves, which is a legitimate generalization because whiskers have linear deflection properties.

For JF25399 the compliance of the flexible object roughly mimics the moment properties of the no-go position 4 mm from the medial border of the no-go range. This is true not only for the binned data but for every day of the experiment (data not shown). The large standard deviations of the curvature in the last data points of the illusion trials are caused by the data of two days (day 5 and day 6) of the experiment. On day 5 a flexible object with particularly low compliance was used, which, when the mouse whisked strongly against it, flexed far enough to result in a 90° curve and stopped to pose resistance to the mouse's whisker. When this point was reached, the curvature accordingly dropped to very low values. On day 6, a more compliant object was used which did not flex as strongly, therefore the curvature values never dropped as strongly. The comparison of illusion curves of the last two days has been plotted in the graph below (**Fig.6.12**). The curvature curves from the fooling trials of the other days resemble the one of day 6. The interday variability of the go and no-go curves can also be seen (**Fig.6.12**).
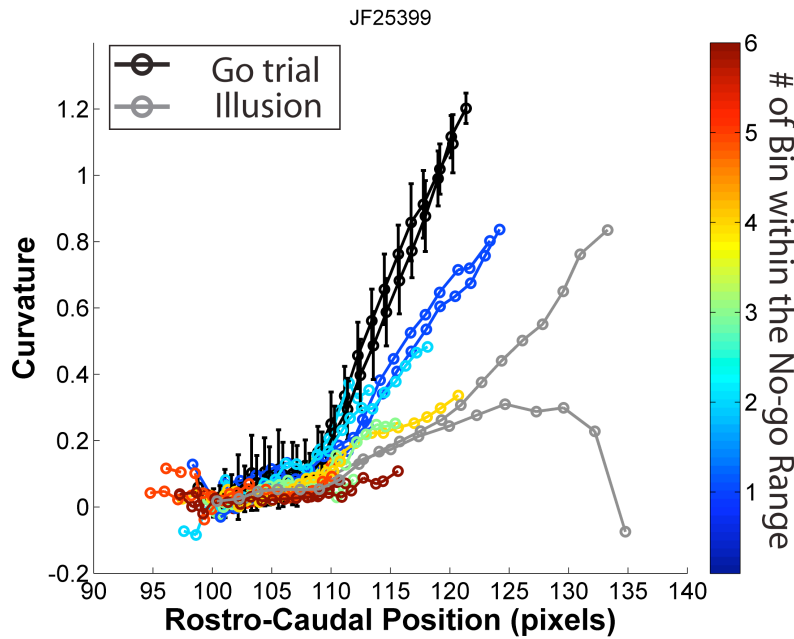
Fig. 6.12: Protraction dependent curvature curves of day 5 and 6 of mouse JF25399 with the same color code as before (***Fig.6.11***). Comparison of variability of curvature in go, no-go and illusion trials. Difference between the two illusion curves resulting from the use of two flexible objects, one with lower compliance (day 5) and one with higher compliance (day 6). Accordingly strong drop in curvature at strong protractions on day 5 compared to the relatively constant curvature trend on day 6. Errorbars are not shown for no-go curves and fooling curves, for clarity purposes.

The graph of mouse JF25395 (***Fig.6.11***) shows the curvature in the fooling trials to be not quite as well placed within the no-go range as for mouse JF25399. This is a result of the data of one day (day 1), where the curvature values of the initial part (before the rising phase) of the fooling curve do not match what is seen in the rising phase (***Fig. 6.13*** left graph). In the initial part of the curve 2 data points lie above the curve of the go trials but the difference between the two points of the fooling curve and the two closest points on the go curve is smaller than the standard deviation of the respective points and therefore not significant (First point (x = 95): mean difference = 0.027, sd(fooling) = 0.039, sd(go) = 0.075; second point (x = 96): mean difference = 0.063, sd(fooling) = 0.069, sd(go) = 0.092). The fooling curve falls below the 1 mm bin of the no-go range at about 103 pixels of rostro-caudal position which is after the beginning of the rising of the curves. On days 3 and 4 (***Fig.6.13*** right graph) the curvature curve of the fooling trials never lies above all other curves.
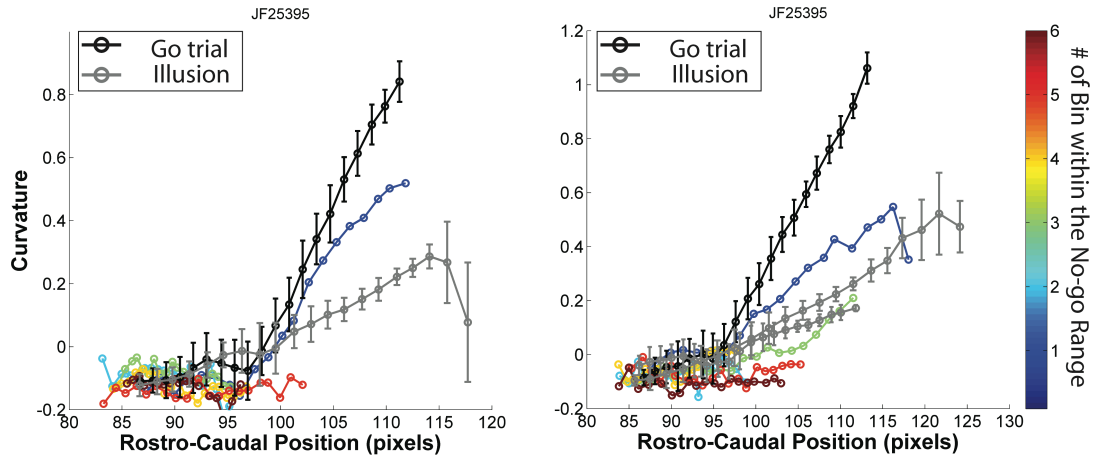
Fig. 6.13: Protraction dependent curvature plot of day 1 (left graph) and both day 3 (shorter grey curve) and day 4 (longer grey curve, go and no-go curves) (right graph) of mouse JF25395 with the same color code as in the two preceding figures. Graph of day 1 shows 2 points of the curve of the fooling trials (grey) lying above the go curve. On day 3 and day 4 the curves never exceed all other curves.

To summarize, the flexible object has been well chosen to mimic the moment properties of the no-go range for mouse JF25399. For mouse JF25395, the flexible object also seems to mimic the moment of the no-go range but on day 1 of the experiment, the curve does not show the desired moment in the initial part of the curve. Therefore one should be more careful when interpreting the behavioral data of this day.

After analyzing the moment perceived by the mice in the fooling trials, the question remains how they interpret this stimulus. The behavior of all 5 mice has been plotted to show the performance of the mice for every trial type over the whole experiment and the number of trials of each type (**Fig.6.14**). All five mice perform the task with high accuracy as can be seen by the percentage of licking in go trials (JF25395 = 92%, JF25397 = 96%, JF25399 = 96%, JF25401 = 95%, JF25403 = 90%) and in no-go trials (JF25395 = 6%, JF25397 = 15%, JF25399 = 5%, JF25401 = 9%, JF25403 = 10%) resulting in an overall performance of ≥90% for every mouse. The mice are expert performers and make very few mistakes when judging object location and their performance did not vary significantly between days (data not shown). Looking at the performance levels one can see that all mice lick in the majority of the fooling trials (JF25395 = 97%, JF25397 = 72%, JF25399 = 74%, JF25401 = 52%, JF25403 = 88%). JF25395 and JF25403 do not perform significantly different in the fooling trials than in the go trials (two sided binomial test, JF25395: p = 0.36; JF25403: p = 1.00). JF25399 and JF25397 show in the fooling trials significantly different (two sided binomial test,

both: p < 0.0001) ~20% lower licking rates than in the go trials, but still lick in a large majority of the fooling trials. JF25401 shows obviously decreased (-43%) and significantly different licking rates (two sided binomial test, p < 0.0001) even though this mouse licks in a slight majority of the trials too. All mice performed in the fooling trials significantly different than in the no-go trials (two sided binomial test, all: p < 0.0001).
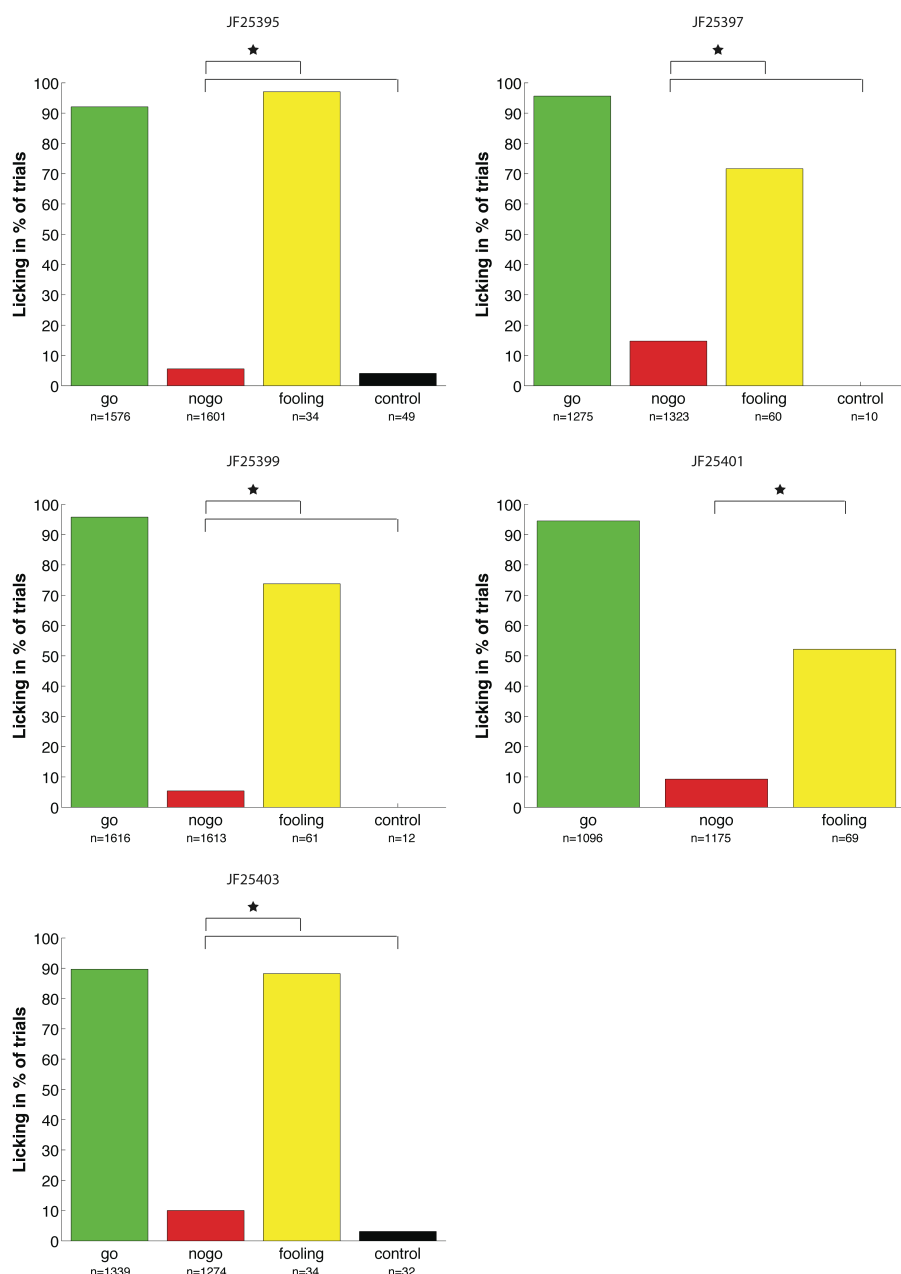
Fig.6.14: Graphs comparing performance levels of all mice (from left to right, top to bottom: JF25395, JF25397, JF25399, JF25401, JF25403) in go (green), no-go (red), fooling (yellow) and fooling control trials (black). For every mouse the number of trials in every trial category is indicated below each bar. Whiskers indicate performances tested for significant difference with a two-sided binomial test. Stars indicate significant difference between the performance levels, no star indicates not significantly different.

From this performance one can conclude that the mice are not fooled into interpreting the flexible object as a no-go stimulus, for which two possible explanations exist. Either the mice perceive the object as a go stimulus and perform accordingly, or they perceive it as a third category and after learning that this third stimulus category is neither rewarded nor punished, they behave according to preference.

To falsify one of the two hypotheses a control experiment was conducted which consisted of dropping a flexible object in a subset of trials (fooling control trials) into the middle of the no-go range (for description see methods section). If the latter explanation would be correct, the mice should show similar performance levels in the fooling control trials as in the fooling trials. The control experiment could only be conducted for one session with mice JF25397 and JF25399 as both of the mice lost their principal whisker after the first day of this control experiment. This is the reason for the low number of control trials performed by these two mice (JF25397 = 10 trials, JF25399 = 12 trials). JF25401 could not participate in the control experiment at all, because also this mouse lost the principal whisker prior to the planned control session. JF25395 and JF25403 however, were performing for 3 days on the control experiment (JF25395 = 49 trials, JF25403 = 32 trials).

Even though just as the fooling trials, these fooling control trials were unrewarded and unpunished the 4 mice licked (percentage of fooling control trials with licking: JF25395 = 4%, JF25397 = 0%, JF25399 = 0%, JF25403 = 3%) at statistically not significantly different rates than seen in the no-go trials (two sided binomial test, JF25395: $p = 0.77$; JF25397: $p = 0.23$; JF25399: $p = 1.00$; JF25403: $p = 0.25$), but significantly different than in the go trials and fooling trials (two sided binomial test, all: $p < 0.0001$). The mice therefore showed the same behavior as in the no-go trials and most probably treated the flexible object in the no-go position just like a no-go stimulus.

This performance makes it likely that the mice interpret the flexible object like the metal pole according to its position, refuting the second explanation. Also the observation that the mice showed roughly the same performance in trials with the flexible object throughout all sessions in the fooling experiment and in the control experiment, displaying no signs of learning of a new stimulus, makes it unlikely that they perceived the flexible object as a third stimulus category.

The behavior in the fooling trials can therefore also be taken as a genuine position judgement and from the performance of the mice one can conclude that the mice are not fooled by the flexible object and interpret it in a majority of the trials as a go

stimulus. With regard of the curvature analysis of mouse JF25399 and JF25395, the results of this experiment show that moment or rate-of-change of moment is not the dominant cue used by this batch of mice for head fixed single whisker radial distance judgement.

6.5. Control Experiment

To be certain that mice do not use other cues than somatosensory perception, three mice (JF25397, JF25399, JF25401), after loosing their principal whisker, performed on a 5 mm radial distance discrimination task while keeping all other features of the behavioral setup constant.

The mice perform radial distance discrimination with high accuracy (mean fraction correct = 91%) with one whisker (C2). Without any whiskers they perform not significantly different from chance levels (mean fraction correct = 50.4%, one sided binomial test, p = 0.28) (***Fig.6.15***). This shows that the task is indeed whisker dependent and the mice did not use other cues besides somatosensory ones to solve the task.
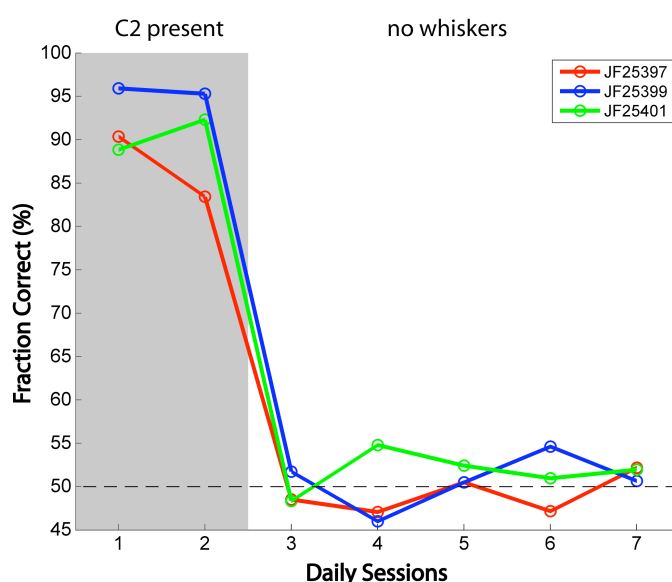

Fig. 6.15: Figure showing the performance of three mice, JF25397 (red), JF25399 (blue), JF25401 (green) on the radial distance discrimination setup for the first two days in possession of their principal whisker C2 (grey background) and for 5 days after loosing the principal whisker (white background). Broken line indicating chance performance.

7. Discussion

The experiments described in the context of this thesis show that mice prefer to use labelled line strategies to discriminate positions differing in their position along the medio-lateral axis in a head fixed behavioral setup. It further shows that mice can make pure radial distance discriminations with one whisker and gives evidence that mice neither use rate-of-change of moment nor azimuthal cues to do so.

7.1. Strategies for Radial Distance Discrimination with Multiple Whiskers

This experiment was designed to find out if mice, when given the choice, prefer to discriminate radial distances via a whisker length dependent mechanism or by extracting the necessary information by an alternative mechanism such as monitoring rate-of-change of moment differences, axial forces or azimuthal cues. Furthermore the experiment gives some hints about the importance of integration over multiple whisker in contrast with monitoring a single whisker which carries enough information to solve the task (labeled line strategy).

This was achieved by trimming two whiskers (B2, D2) so that they could only contact the object in the go position but not in the no-go position. ~8 other whiskers could contact both positions (for description see methods chapter). The mice could choose between several strategies to solve the task: with a binary labelled line strategy the mice could choose to only monitor B2 or D2. By monitoring contact versus no contact of B2/D2 and turning the radial distance discrimination into a binary task, the mouse would solve the radial discrimination task as a detection task. If mice would choose to adopt this strategy, one should see a significant performance drop when trimming B2/D2.

Another possibility is that mice prefer to solve radial distance tasks with cues other than contact / no contact, for which the trimming of B2 and D2 should have a small impact on the performance as multiple whiskers remain to sense the stimulus.

As trimming of B2 leads to a significant drop of performance levels to only slightly above chance performance, this experiment shows that mice choose to adopt a binary

strategy and monitor B2 contact. D2 trimming did not have a significant impact on performance.

The finding in aperture discrimination that every whisker is equally important (Krupa et al., 2001) is therefore not true in head fixed medio-lateral distance discrimination, as one should see only small impacts on performance, both for trimming D2 and for trimming B2 (with a whiskerfield of ~10 whiskers a drop of ~10% for cutting D2 and ~11% for trimming B2) if every whisker would be equally important for the mouse.

If integration over multiple whiskers would be necessary in medio-lateral distance discrimination as proposed from aperture discrimination experiments (Krupa et al., 2001), the mice would try to contact the pole with both D2 and B2 and learn to rely on contact information from both whiskers. Cutting D2 and B2 should therefore result in a roughly equally large performance drop. Therefore integrating stimuli of multiple whiskers does not seem to be the primary goal of the mice to perform position discriminations, as the drop in performance after D2 trimming versus B2 trimming was significantly different.

Further analysis could be based on whisker tracking to see whether mice try to minimize contacts with whiskers other than B2. Correlating whisker contacts in trials with the resulting response (correct or incorrect) could give information about the processing of information of individual whiskers. For example should one see that in a significant number of no-go trials in which the mice contact the stimulus with C2, they respond incorrectly by licking, it could be concluded that it is hard for mice to selectively monitor individual whiskers. This would have implications for our understanding of information integration over multiple whiskers.

7.2. Radial Distance Experiment

In this experiment it was addressed whether or not mice can do pure radial distance discrimination.
As pointed out in the results chapter, the relative difference in position of the clusters in the 2 dimensions (*Fig.6.6*) can most probably not be reliably detected by the mice, for which the same arguments hold true as for discarding the hypothesis of the azimuthal difference alone as a means to discriminate between go and no-go. Another reason

why the relative cluster position can not be a cue for the discrimination is that within the course of the experiment the coordinates of go and no-go position had to be fine tuned many times from one day to the next and sometimes even within the same day to insure small azimuthal differences between go and no-go. When changing the coordinates in many cases the relative position of the clusters was inverted but caused no impact on the discrimination performance, as would be expected when the relative position of the clusters would be the cue for solving the task.

The second potential azimuthal cue is the difference in azimuthal spread of multiple whisker contacts. This azimuthal spread is larger in go trials which is probably due to trigonometric rules. But also the fact that the mice are licking in the go trials, an activity that is tightly coupled with heavy whisking, could be an explanation for the larger spread, as it is possible that whisking while licking and discriminatory whisking are different modes of whisking. The same arguments can be used to attempt an explanation for the difference in azimuthal shift within single go trials versus no-go trials.

Therefore, it was asked whether the in some trials considerable azimuthal spread could be used as an azimuthal cue for radial distance discrimination. From the histograms (*Fig.6.9*) it can be concluded that the azimuthal spread does not exceed the supposed detection limit of 4° in a majority of trials. Additionally, there is a bias towards measuring larger position deviations than actually present. This arises from the limitations of capturing the actual initial contact, as what can be tracked is only the first frame in which contact becomes visible. In some cases when mice whisk with high velocities (>1400 deg/sec), the angular change in consequent frames can be substantial even with recording rates of 1000 Hz. These facts makes it unlikely that azimuthal-translational spread is a potent cue on which the mice rely heavily to solve the task. The spread of azimuth and translation of the whisker at contact is more likely to be a non-relevant feature of whisking than an active mechanism critical for position judgement.

Still, further experiments should be directed towards addressing the question whether or not the integration of the relative distribution of translation and azimuth over multiple contacts is a cue used by the animals. In this thesis it could only be argued that these cues are most probably not used because of azimuthal discrimination thresholds drawn from psychometric curves obtained through experiments by Daniel O'Connor. An

experiment particularly designed to give ultimate answers about the importance of this distribution would be preferable. This could be achieved by training the mice on a radial distance discrimination task and then sectioning the facial nerve which controls the extrinsic muscles. As the extrinsic muscles are responsible for moving the whisker pad and therefore control translation, a section would abolish translational movements. This would lead to a situation where the mice would only be able to whisk via the use of their intrinsic muscles bringing about azimuthal changes but changing the translation-azimuth distribution would not be possible. The azimuthal spread over multiple contacts would therefore be abolished. The impact of this procedure on the performance of the mice would then give information about the importance of the translational-azimuthal spread for judging radial distance. A control experiment where the same procedure is applied to mice which are trained to judge distances in the anterior posterior axis would have to be conducted if there is a significant drop of performance levels, to insure that the effect is task specific and not a general impairment of somatosensory perception.

7.3. Psychometric Curves

The psychometric curve experiment shows that mice can detect radial distance offsets as small as 1 mm, although only slightly above chance. 2 mm can be detected reliably.

In this experiment further effort could be invested in whisker tracking. This would allow to characterize if mice try to maximize some whisking variables in the sessions with small radial distances between go and no-go stimulus. Also one could analyze whether there are correlations between specific modes of whisking and errors in performance. These correlations could potentially hold important information about the sensory variables used as cues for radial distance discrimination.

7.4. Fooling Experiment

The question addressed in this experiment, which mechanical variables are used for radial distance discrimination, has to be further investigated. The experiment falsified the hypothesis that mice use moment or rate-of-change of moment as the mechanical cue for radial distance judgement but which variable is in fact used remains to be answered.

One possible strategy would be for the mice to monitor axial forces. These are the forces in the medio-lateral direction, in other words pushing the whisker into the follicle, and they differ depending on the distance of the object from the follicle. This force difference has the same origin as the moment differences. As the whisker is a tapered rod it bends more heavily the more lateral the whisked against object is located. This results on the one hand in a smaller force (moment) but also in a shift of the direction of this force. Both phenomena have been schematized (*Fig.7.1*) in form of two arrows (resembling force vectors) indicating force magnitude through length of the vector and force direction through direction of the force vectors.
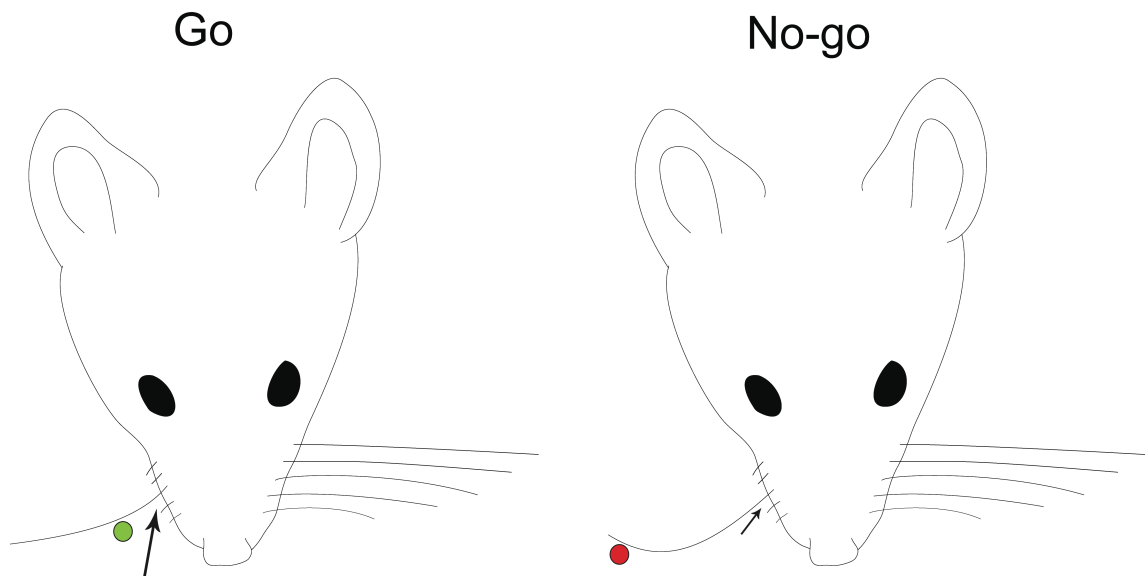
Fig. 7.1: Schematic drawing of the whisker shape upon contact with an object located relatively close to the follicle (left drawing) and one relatively distal (right drawing). Length of the arrows indicates force magnitude, direction of the arrow indicates force direction. These values do not represent true force values, but should indicate an expected trend.

When the object is located close to the follicle, it contacts the whisker in a position where the whisker is relatively thick. When hitting and pushing the whisker against this object, the whisker does not bend heavily and the major portion of the resulting force is therefore directed against the posterior part of the follicle wall. On the other hand when encountering an object that is located more laterally, a position on the whisker is in contact where the whisker is thinner. This leads to a strong flexing of the whisker, resulting in a situation where a large portion of the force is directed onto the medial part of the follicle sac.

This hypothesis should be tested by measuring the deflection properties of the whiskers similarly as has been done for the rat's whisker (Birdwell et al., 2007) and then modeling the force distributions for different radial contact situations. Also empirical testing should be pursued by training the mice on a single whisker radial distance discrimination task and during the course of one session altering the stiffness properties of their principal whisker by using an agent that enhances the compliance (e.g. hairspray). This should result in smaller curvature of the whisker at contact and therefore in a significant drop in performance levels if the mice use axial forces to judge radial distance. In combination with control experiments where mice are trained on an azimuthal discrimination task and also treated with the same whisker compliance modulation procedure, to make sure that the impact on performance is task specific and not an unspecific impairment of somatosensation, this would provide a means of testing whether or not axial forces are an important cue for radial distance discrimination.

Another not perfectly resolved problem with significance to all experiments evolves around the issue of whisker tracking. As it is still not possible to have a fully automated tracker tracking the movies and afterwards extracting with perfect precision events of contacts, it remains necessary to manually track the movies frame by frame. This is laborious and accounts for the fact that only movies of two mice were tracked for the fooling experiment. It would be preferable to track whiskers of additional mice participating in the fooling trials to have further examples of curvature curves for the three trial types.

8. Appendix

8.1. Matlab Codes

8.1.1. Radial Discrimination of Two Positions

```matlab
function [x,y,interX,interY,reward] = ChooseNextPosition(obj,trialNumber)
% Function receives coordinates for two positions and on each call pseudo
% randomly returns one of them, an intermediary position and the reward
% identity of the position.
% by Lorenz Pammer, March 2009

persistent positionList;
persistent rewardList;
persistent interPosition;


%%
if trialNumber == 1

    disp('Behavioral Task: Discrimination of two user defined positions.');

    goX = input('Define the Motor1 position of the go-position. ');
    goY = input('Define the Motor2 position of the go-position. ');
    nogoX = input('Define the Motor1 position of the nogo-position. ');
    nogoY = input('Define the Motor2 position of the nogo-position. ');

    go = [goX;goY];
    nogo = [nogoX;nogoY];
    connection = nogo - go;

        for i = 1 : 1000 % creates a list of 2000 positions
            positionList(:,i) = go;
            positionList(:,i+1000) = nogo;
        end
        arb = randperm(length(positionList)); % arbitrary list of numbers for every element in
positionList
        positionList = positionList(:,arb); % randomizes positionList entries


    for h = 1 : length(positionList)
        rewardList(h) = all(positionList(:,h) == go); % produces vector with 1 for each go, 0 for
each nogo
    end

    goRepeat = strfind(rewardList,[1 1 1 1]); % finds repeats of 4 or more go positions
    nogoRepeat = strfind(rewardList,[0 0 0 0]); % finds repeats of 4 or more nogo positions

    positionList(:,[goRepeat nogoRepeat]) = []; % removes the found repeats in positionList
    rewardList([goRepeat nogoRepeat]) = []; % removes the found repeats in rewardList
    rewardList = single(rewardList);

    interPosition = round(go + (connection/2)); % intermediary position is midway between go & nogo

end


trialPosition = round(positionList(:,trialNumber)); % defining output
reward = rewardList(trialNumber);
x = trialPosition(1);
y = trialPosition(2);
interX = interPosition(1);
interY = interPosition(2);

end
```

8.1.2. No-go Range with Unrewarded Go Position

```matlab
function [x,y,interX,interY,reward] = ChooseNextPosition(obj,trialNumber)
% Arbitrary nogo positions between 2 specified border points
%
% Function receives coordinates for a go position and the two positions
% between which the nogo positions should be placed. On each call it
% returns either the go or a arbitrarily chosen nogo, an intermediary
% position and the reward identity of the position.
%
% by Lorenz Pammer, April 2009

persistent positionList;
persistent rewardList;
persistent interPosition;
persistent medial;
persistent lateral;
persistent range;
persistent go;


%% Input of user defined Positions

if trialNumber == 1

    disp('Behavioral Task: Discrimination of a user defined go and a user defined distributed
nogo.');

    goX = input('Define the Motor1 position of the go-position. ');
    goY = input('Define the Motor2 position of the go-position. ');
    medialX = input('Define the Motor1 position of the medial border. ');
    medialY = input('Define the Motor2 position of the medial border. ');
    lateralX = input('Define the Motor1 position of the position of the lateral border. ');
    lateralY = input('Define the Motor2 position of the position of the lateral border. ');

    go = [goX;goY];
    medial = [medialX;medialY];
    lateral = [lateralX;lateralY];
    range = lateral - medial;

    %% Create list of positions
    a = 1000*0.0625; % adjusting for lost go positions due to rewardless gos
    a = round(1000+a);

    for i = 1 : a % creates a list of 2126 positions
        positionList(:,i) = go; % writes go positions to positionList
        positionList(:,i+a) = round(medial + (range*rand)); % randomly generates positions between
the specified nogo boundaries
    end
    positionList(:,2001:length(positionList)) = []; % deletes 126 nogo Positions

    arb = randperm(length(positionList)); % produces matrix of arbitrary numbers for every element of
positionList
    positionList = positionList(:,arb); % randomizes positionList


    for h = 1 : length(positionList)
        rewardList(h) = all(positionList(:,h) == go); % renders vector with 1 for each go, 0 for each
nogo
    end

    goRepeat = strfind(rewardList,[1 1 1 1]); % finds repeats of 4 or more go positions
    nogoRepeat = strfind(rewardList,[0 0 0 0]); % finds repeats of 4 or more nogo positions

    positionList(:,[goRepeat nogoRepeat]) = []; % removes the found repeats in positionList
    rewardList([goRepeat nogoRepeat]) = []; % removes the found repeats in rewardList
    rewardList = single(rewardList);

    for i = 33  : 16 : (length(positionList)-50) % one unrewarded (reward identity=2) go randomly in
every 16 positions
        arb = randperm(15);
        arb = arb(1);
        if rewardList(arb+i) == 1 % if arbitrarily chosen position is a go
            rewardList(arb+i) = 2; % change reward identity to unrewarded/unpunished 2
        else
            changed = 0;
            for j = 1 : 8
                if changed == 0 % look in the following trials for a go
                    if rewardList(arb+i+j) == 1
                        rewardList(arb+i+j) = 2; % change reward identity
                        changed = 1;
                    end
                end
            end
        end
```

```matlab
        end
    end


end

%% Choosing intermediary position

whisker = lateral - go;
interPosition = round(go + (whisker*rand)); % chooses pseudoarbitrarily
% a position between go and the lateral border as the intermediary position

%% Defining Output

trialPosition = round(positionList(:,trialNumber));
reward = rewardList(trialNumber);
x = trialPosition(1);
y = trialPosition(2);
interX = interPosition(1);
interY = interPosition(2);

end
```

8.1.3. Illusion Task

```matlab
function [x,y,interX,interY,reward] = ChooseNextPosition(obj,trialNumber)

% Somatosensory Illusion
%
% Function receives coordinates for a go position, two positions
% between which the nogo positions should be placed and the position at
% which the illusion object is located in the go position. On each call it
% returns one of them, an intermediary position and the reward identity of
% the position.
%
% by Lorenz Pammer, April 2009


persistent positionList;
persistent rewardList;
persistent interPosition;
persistent medial;
persistent lateral;
persistent range;
persistent illusion;
persistent go;

%% Input of user defined Positions

if trialNumber == 1

    disp('Behavioral Task: Discrimination of a go position, nogo position distribution and an illusion.');

    goX = input('Define the Motor1 position of the go-position. ');
    goY = input('Define the Motor2 position of the go-position. ');
    medialX = input('Define the Motor1 position of the medial boundary. ');
    medialY = input('Define the Motor2 position of the medial boundary. ');
    lateralX = input('Define the Motor1 position of the position of the lateral boundary. ');
    lateralY = input('Define the Motor2 position of the position of the lateral boundary. ');
    illusionX = input('Define the Motor1 position of the position of the illusion. ');
    illusionY = input('Define the Motor2 position of the position of the illusion. ');


    go = [goX;goY];
    medial = [medialX;medialY];
    lateral = [lateralX;lateralY];
    range = lateral - medial;
    illusion = [illusionX;illusionY];

    %% Create list of positions

    a = 1000*0.0312; % adjusting for lost go positions due to rewardless gos
    a = round(1000+a);
    for i = 1 : a % creates a list of 2062 positions
        positionList(:,i) = go; % writes 1031 go positions to positionList
        positionList(:,i+a) = round(medial + (range*rand)); % randomly generates 1031 positions
between the specified nogo boundaries
    end
    positionList(:,2001:length(positionList)) = []; % deletes 62 nogo Positions
```

```matlab
    arb = randperm(length(positionList)); % produces matrix of arbitrary numbers for every element of
positionList
    positionList = positionList(:,arb); % randomizes positionList


    for h = 1 : length(positionList)
        rewardList(h) = all(positionList(:,h) == go); % renders vector with 1 for each go, 0 for each
nogo
    end

    goRepeat = strfind(rewardList,[1 1 1 1]); % finds repeats of 4 or more go positions
    nogoRepeat = strfind(rewardList,[0 0 0 0]); % finds repeats of 4 or more nogo positions

    positionList(:,[goRepeat nogoRepeat]) = []; % removes the found repeats in positionList
    rewardList([goRepeat nogoRepeat]) = []; % removes the found repeats in rewardList
    rewardList = single(rewardList);

    for i = 33 : 32 : (length(positionList)-50)
        arb = randperm(31);
        arb = arb(1);
        positionList(:,arb+i) = illusion; % within every 33 trials one illusion is pseudoarbitrarily
placed
        rewardList(arb+i) = 2; % rewardList is changed to contain illusion identity 2
    end

    for i = 33  : 32 : (length(positionList)-50) % one unrewarded (reward identity=2) go randomly in
every 32 positions
        arb = randperm(31);
        arb = arb(1);
        if rewardList(arb+i) == 1 % if arbitrarily chosen position is a go
            rewardList(arb+i) = 2; % change reward identity to unrewarded/unpunished 2
        else
            changed = 0
            for j = 1 : 8 % look in the following trials for a go
                if changed == 0
                    if rewardList(arb+i+j) == 1
                        rewardList(arb+i+j) = 2; % change reward identity
                        changed = 1;
                    end
                end
            end
        end
    end
end

%% Choosing intermediary position
% called each trial

whisker = lateral - go;
inter = round(go + (whisker*rand)); % chooses a pseudoarbitrary position between go and the lateral
% border as the intermediary position
interList = [illusion inter];

if trialNumber == 1 % statement deletes current trialPosition from interList

    if all(interList(:,1) == positionList(:,trialNumber))
        interList(:,1) = [];
    elseif all(interList(:,2) == positionList(:,trialNumber))
        interList(:,2) = [];
    end

else % statement deletes current and previous trialPosition from interList

    if all(interList(:,1) == positionList(:,trialNumber)) || all(interList(:,1) ==
positionList(:,trialNumber-1))
        interList(:,1) = [NaN;NaN];
    end
    if all(interList(:,2) == positionList(:,trialNumber)) || all(interList(:,2) ==
positionList(:,trialNumber-1))
        interList(:,2) = [NaN;NaN];
    end

end

interList(:,find(isnan(interList(1,:)))) = []; % deletes previously found and NaN marked positions of
interList
n = size(interList);
if n(2) == 2
    arb = round(1.3+rand); % 1:5 chance of illusion position as intermediary position
else
    arb = randperm(length(interList(1,:)));
end

interPosition = interList(:,arb(1)); % pseudoarbitrarily chooses one of the two not current positions
as intermediary
```

```matlab
%% Defining Output

trialPosition = round(positionList(:,trialNumber));
reward = rewardList(trialNumber);
x = trialPosition(1);
y = trialPosition(2);
interX = interPosition(1);
interY = interPosition(2);


end
```

8.1.4. Fooling Control Experiment

```matlab
function [x,y,interX,interY,reward] = ChooseNextPosition(obj,trialNumber)

% Control with flexible object in nogo range
%
% Function receives coordinates for a go position, two positions
% between which the nogo positions should be placed and the position at
% which the flexible object is located in the middle of the nogo range. On
% each call it returns one of them, an intermediary position and the reward
% identity of the position.
%
% by Lorenz Pammer, May 2009


persistent positionList;
persistent rewardList;
persistent interPosition;
persistent medial;
persistent lateral;
persistent range;
persistent illusion;
persistent go;

%% Input of user defined Positions

if trialNumber == 1

    disp('Behavioral Task: Discrimination of a go position, nogo position distribution and an
illusion.');

    goX = input('Define the Motor1 position of the go-position. ');
    goY = input('Define the Motor2 position of the go-position. ');
    medialX = input('Define the Motor1 position of the medial boundary. ');
    medialY = input('Define the Motor2 position of the medial boundary. ');
    lateralX = input('Define the Motor1 position of the position of the lateral boundary. ');
    lateralY = input('Define the Motor2 position of the position of the lateral boundary. ');
    illusionX = input('Define the Motor1 position of the position of the illusion. ');
    illusionY = input('Define the Motor2 position of the position of the illusion. ');


    go = [goX;goY];
    medial = [medialX;medialY];
    lateral = [lateralX;lateralY];
    range = lateral - medial;
    illusion = [illusionX;illusionY];

    %% Create list of positions

    %    a = 1000*0.0312; % adjusting for lost go positions due to rewardless gos
    %    a = round(1000-a);
    for i = 1 : 1000 % i creates a list of 2000 positions
        positionList(:,i) = go; % writes 1000 go positions to positionList
        positionList(:,i+1000) = round(medial + (range*rand)); % randomly generates 1031 positions
between the specified nogo boundaries
    end
    %    positionList(:,2001:length(positionList)) = []; % deletes 62 nogo Positions
    %

    arb = randperm(length(positionList)); % produces matrix of arbitrary numbers for every element of
positionList
    positionList = positionList(:,arb); % randomizes positionList


    for h = 1 : length(positionList)
        rewardList(h) = all(positionList(:,h) == go); % renders vector with 1 for each go, 0 for each
nogo
    end

    goRepeat = strfind(rewardList,[1 1 1 1]); % finds repeats of 4 or more go positions
    nogoRepeat = strfind(rewardList,[0 0 0 0]); % finds repeats of 4 or more nogo positions
```

```matlab
        positionList(:,[goRepeat nogoRepeat]) = []; % removes the found repeats in positionList
        rewardList([goRepeat nogoRepeat]) = []; % removes the found repeats in rewardList
        rewardList = single(rewardList);

        for i = 33 : 32 : (length(positionList)-50)
            arb = randperm(31);
            arb = arb(1);
            positionList(:,arb+i) = illusion; % within every 33 trials one illusion is pseudoarbitrarily
placed
            rewardList(arb+i) = 2; % rewardList is changed to contain illusion identity 2
        end

        for i = 33  : 32 : (length(positionList)-50) % one unpunished (reward identity=2) nogo randomly
in every 32 positions
            arb = randperm(31);
            arb = arb(1);
            if rewardList(arb+i) == 0 % if arbitrarily chosen position is a nogo
                rewardList(arb+i) = 2; % change reward identity to unrewarded/unpunished 2
            else
                changed = 0
                for j = 1 : 8 % look in the following trials for a nogo
                    if changed == 0
                        if rewardList(arb+i+j) == 0
                            rewardList(arb+i+j) = 2; % change reward identity
                            changed = 1;
                        end
                    end
                end
            end
        end
    end
end

%% Choosing intermediary position
% called each trial

whisker = lateral - go;
inter = round(go + (whisker*rand)); % chooses a pseudoarbitrary position between go and the lateral
% border as the intermediary position
interList = [illusion inter];

if trialNumber == 1 % statement deletes current trialPosition from interList

    if all(interList(:,1) == positionList(:,trialNumber))
        interList(:,1) = [];
    elseif all(interList(:,2) == positionList(:,trialNumber))
        interList(:,2) = [];
    end

else % statement deletes current and previous trialPosition from interList

    if all(interList(:,1) == positionList(:,trialNumber)) || all(interList(:,1) ==
positionList(:,trialNumber-1))
        interList(:,1) = [NaN;NaN];
    end
    if all(interList(:,2) == positionList(:,trialNumber)) || all(interList(:,2) ==
positionList(:,trialNumber-1))
        interList(:,2) = [NaN;NaN];
    end

end

interList(:,find(isnan(interList(1,:)))) = []; % deletes previously found and NaN marked positions of
interList
n = size(interList);
if n(2) == 2
    arb = round(1.3+rand); % 1:5 chance of illusion position as intermediary position
else
    arb = randperm(length(interList(1,:)));
end

interPosition = interList(:,arb(1)); % pseudoarbitrarily chooses one of the two not current positions
as intermediary

%% Defining Output

trialPosition = round(positionList(:,trialNumber));
reward = rewardList(trialNumber);
x = trialPosition(1);
y = trialPosition(2);
interX = interPosition(1);
interY = interPosition(2);


end
```

9. References

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Profile
Born on May 12th 1985 in Vienna, Austria

German - native, English - fluent, French - intermediate, Romanian - beginner

Education
Molecular Biology — University of Vienna, Austria 2004-2009
Completed the first stage of studies, similar to bachelor, with distinction.
Focus on neurobiology, cell biology, immunology & microbiology in the second stage, equivalent to a masters program, finishing with a nine months diploma thesis in the field of neuroscience in Janelia Farm.

ERASMUS — University of Geneva, Switzerland 2008
After being selected as an ERASMUS stipendiary, spent the summer semester following lectures of the "Master in Proteomics and Bioinformatics" and the "Master of Neuroscience".

venture.lab — venture.challenge, Switzerland 2008
Selected by the Swiss organization venture.challenge to participate in venture.lab, a one semester training course in setting up startup companies.

Student Exchange Year — Senator Gershaw High School, Canada 2000-2001
Spent one school year in Bow Island, Alberta, Canada, acquirement of fluent English skills.

High School — Gymnasium Erlgasse Vienna, Austria 1995-2003
English first, Latin second, French third foreign language
Graduation with distinction

Elementary School — De La Salle School Vienna, Austria 1991-1995

Research & Work Experience
Diploma/Master Student Svoboda laboratory, Janelia Farm, U.S.A. 2008-2009
Spending nine months in the laboratory of Karel Svoboda in the HHMI Research Campus Janelia Farm, doing research in the field of mouse somatosensation as a diploma thesis. Psychophysical experiments to define properties of radial distance coding in the whisker system and 3D-reconstruction of whisker movement to quantify whisker rotation.

Intern Dickson laboratory, IMP Vienna, Austria 2008
In order to learn programming with MATLAB, spent two months coding a program for analyzing Drosophila feeding behavior, by adapting an existing fly tracker and creating a code, which detects and analyzes feeding events in videos. The resulting program quantifies fly feeding choice.

Intern Wurm laboratory, EPFL Lausanne, Switzerland 2008
To get an insight into applied science and more experience with cloning, worked for two months during spring on good manufacturing practice compatible transient transfection optimization with Florian Wurm.

Rotations Center For Brain Research of the Medical University of Vienna, Austria 2007
Completed one month of rotations, learning several neuroscience relevant techniques, as part of the elective Neurobiology course in the labs of Michael Kiebler, Jürgen Sandkühler and Werner Sieghart among others.

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Intern Dickson laboratory, IMP Vienna, Austria 2007
Following a growing interest in neurobiology, joined Barry Dickson's group for ten weeks of fascinating fly pushing. Screening for and analysis of genetic factors and neuronal populations influencing Drosophila Melanogaster mating and feeding behavior.

Intern Zenclussen laboratory, Charité Berlin, Germany 2006
After doing laboratory courses in university, joined the lab of Ana Zenclussen for five weeks in summer to engage in non educational laboratory work. Focussing on molecular mechanisms contributing to fetal-maternal tolerance, concentrating on arginase-1 expression patterns.

Civil Servant Sibiu, Romania, 2003-2004
Fulfilling conscription, with fourteen months of civil service, supporting elderly members of the Austrian minority in Sibiu. Thereby acquirement of Romanian language skills.

Intern Rüker laboratory, B.O.K.U. Vienna, Austria 2002
To make sure my love for molecular biology would not fade, when bluntly exposed to the reality of laboratory work, I did a one month vacation job being fascinated by gel electrophoresis.

Stipends
students4excellence Stipendiary, Summer 2009

HHMI - Janelia Scientific Visitors Program, October 2008 - June 2009

ERASMUS Stipendiary, Summer semester 2008

Publications
L Pammer, DH O'Connor, NG Clack, D Huber, T Komiyama, EW Myers, K Svoboda - Mice can discriminate tactile stimuli along their whisker (radial object distance) *SFN 2009*

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