Whisking signals motor preparation and the behavioral state of mice

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Abstract

A central function of the brain is to plan, predict and imagine the effect of movement in a dynamically changing environment. Here we show that the position of the vibrissae, sets of mobile tactile sensors on each side of the face, reflects the behavioral state and predicts the movement of mice, head-fixed in a plus-maze floating on air. Whisker position and whisking as well as nose position signal whether the animal is moving backward or forward, turning right or left, standing still or moving, expecting reward or licking. Surprisingly, the relationship between bilateral whisker position and behavioral state has little to do with tactile input from the whiskers. Thus, in addition to a tactile exploratory function, these mobile sensors on the face of a mouse signal the behavioral and motor preparation state of the animal.

Introduction

One of the principal functions of the brain is to control movement (Llinas, 2001; Wolpert and Ghahramani, 2000; Wolpert and Landy, 2011). According to one view, brains may even have evolved for the sole purpose of guiding and predicting the effect of movement (Llinas, 2001). Whether or not the brain evolved for motor control, it is clear that the activity of many brain circuits is intimately linked to movement (Fetz, 1994) and that movement can involve many sensory-motor modalities. For example, the simple act of reaching to touch an object requires postural adjustments, and movement of the head, eye and limbs, with the eyes often moving first (Barnes et al., 1979; Anastasopoulos et al., 2009).

The rodent whisker system is a multimodal sensory-motor system. While it is often called a model sensory system (Van der loos and Woolsey, 1973), where each whisker is associated with 1000s of neurons in the trigeminal somatosensory pathways, this system is in fact also a model motor system – with single muscles associated with each whisker (Dorfl, 1983; Sachdev et al., 2002; Grinevich et al., 2005; Haidarliu et al. 2013). Not only do mice have the potential to control the motion of these tactile sensors individually, but the motion of the whiskers is often coordinated with motion of the head (Towal and Hartmann 2006; Schroeder and Ritt, 2016). Additionally, whisking can be triggered by sniffing, chewing, licking and walking (Welker 1964; Deschenes et al., 2012; Sofroniew et al., 2014). Whisking can also be used to detect the location of objects, it can be used socially, and it can even predict head direction (Krupa et al. 2004; Sellien et al. 2005; Knutsen et al. 2006; Godde et al. 2010; Cao, et al., 2012; Grant et al. 2012a,b; Arkley et al. 2014; Reimer et al., 2014; Saraf-Sinik et al. 2015; Voigts et al. 2015; Lenschow et al., 2015). Even though a lot is known about whisker use,

most earlier studies have limited their observations to simple preparations; whisker tracking in settings in which rodents move through environments that have walls, contours and textures is rare.

The development of virtual reality systems has increased the complexity of behaviors used in head-fixed rodents. However, virtual systems are predominantly geared toward creating virtual visual worlds around animals (Holscher et al. 2005; Harvey et al., 2009). An alternative to the virtual visual mazes is to use floating platforms presenting 'real-world' environments, physical environments that mice can touch and feel. Mice can be placed in complex settings with walls, and textures where they learn to navigate actual mazes (Nashaat et al., 2016). While it is well known that tactile input from whiskers is essential to the sensory world that mice create around themselves, the role of whiskers and whisker movement in motor preparation and motor planning is unknown. Here we show that in some contexts whisking has little to do with tactile input, instead whiskers can signal the behavioral and "movement state" of the animal.

Methods

We performed all procedures in accordance with protocols approved by the Charité Universitätsmedizin Berlin and the Berlin Landesamt für Gesundheit und Soziales (LAGeSo) for the care and use of laboratory animals.

Surgery. Five adult male mice, weighing 25-32g were used in these experiments. Animals were anesthetized with ketamine/xylazine (90 mg/kg ketamine and 10 mg/kg xylazine) to prepare them for head-fixation. A lightweight aluminum head post was attached using a mixture of Rely-X cement and Jet acrylic black cement. Animals were monitored during recovery and were given antibiotics (Enroflaxacin) and analgesics (Buprenorphine and Carprofen).

Air Track Plus maze. The details of the custom-made plus maze, air-table and monitoring system for detecting the location and position of the plus maze have been published previously (Nashaat et al., 2016). Here we used a clear plexiglass air table mounted on aluminum legs, on which we placed a 3D printed circular platform 30 cm in diameter, that was shaped into a plus maze, where each lane of the maze was 10 cm long, 4 cm wide and 3 cm high, and the center of the maze, the "rotation area" was 10 cm in diameter (**Figure 1A**). The walls of the lanes had different textures. Some lanes had smooth walls others had walls with vertical evenly spaced raised indentations. Mice were not trained to discriminate between the different textures of the walls, they were simply exposed to them.

A pixy camera / Arduino interface tracked the Air-floating maze position with 35 fps, triggering an actuator that moved the reward spout toward the animal (**Video 1**), when the animal entered the correct lane.

Training. A week after surgical preparation, animals were gradually habituated to handling and head fixation on the plus maze. Mice were kept under water restriction and were monitored daily to ensure a stable body weight not less than 85% of their initial starting weight. Habituation consisted of head fixing mice in the plus maze, while manually giving condensed milk as a reward. Subsequently, over the next few days animals were guided manually with experimenter nudging and moving the maze under the mouse. Mice learned to rotate the maze, go forward and backward, collecting a reward at the end of a specific lane. The rewarded lane was indicated by an LED (placed at the end of the lane) turning off once the animals were facing the correct lane (**Figure 1A**).

A single complete trial started when the LED turned on, the animal walked backwards to the center of the maze, rotated the maze, orienting itself to the correct lane (indicated by the LED turning off). Then the animal moved forward to the end of this lane, waited for the lick tube to descend and licked the tube for a reward (**Video 1,2**). Note that a trial could last indefinitely, there was no requirement for the animal to move the maze quickly, or even to keep the maze moving. Thus, individual trials could vary widely in their duration.

Data acquisition began once animals performed ~50 trials in a day. Each day, the same two whiskers on each side of the face and a small spot on the nose were painted red or green using UV Glow 95 body paint (F**igure 1B, left**). The tips of the tracked whiskers were trimmed to ensure that they stayed within the 4 cm width of the lane. All other whiskers were left intact. High speed video was acquired at 190 Hz, with a Basler Camera while the set up was illuminated with two dark lamps. Data was acquired in dark light conditions, where the UV glow colors on whiskers were most clearly visible and distinguishable from the background (**Figure 1B**).

Data selection and image analysis

We used 3 days of data from 5 well trained animals. These mice performed 50-100 trials in a single hour-long session. Data used here was from animals that could move the maze smoothly. Trials were selected for analysis if the whiskers were visible, the paint was glowing uniformly, and if in the course of the trial, the view of the whiskers was not obstructed by the motion of the animal (Nashaat et al., 2017).

Behavioral states were annotated manually by marking the frames when state transitions occurred. The time point of entry into or exit from the lane was determined by using the position of the eyes, in relationship to the edges of the lanes. The frame on which the animal started moving continuously in one direction was defined as the onset of forward or backward movement.

Data were acquired as Matrox-format video files, each file covering a single trial. These files were converted into H.264-format using ZR view (custom software made by Robert Zollner, Eichenau, Germany). Maximum intensity projections of painted whiskers and nose position within one session were created using Image J. From the maximum intensity projection, three individual rectangular regions of interest (ROI) were selected using the rectangular selection tool in ImageJ. Two ROIs included whiskers, on each side of the face, and a third one was set around the nose (**Figure 1B, right**). The ROI dimensions were calculated using the Measurement tools in ImageJ.

Tracking

Whisker and nose position were tracked for each session, and for each animal separately. A custom-made ImageJ plugin (https://github.com/gwappa/Pixylator, version 0.5) or an equivalent Python code (https://github.com/gwappa/python-videobatch, version 1.0) was used to track the pixels

inside the ROI selected with ImageJ. For each frame, the pixels that belonged to a particular hue value (red or green) were collected and the center of mass for the pixels was computed using the brightness / intensity of the pixel. If tracking failed in some frames, i.e. the algorithm failed to detect any matched pixels – because of shadows, movement, or the whisker getting bent under the animal or against a wall – these frames were dropped and linear interpolation was used to ascribe position values in the missing frames. From this analysis, we created masks for each whisker (**Video 3**), that tracked the whisker position for the entire session.

Analysis

Asymmetry of whisker position was assessed on a trial by trial basis, using values of normalized positions, which made it possible to compare side to side differences in position. In each trial, the most retracted and the most protracted positions became 0 and 1, respectively. Set points and amplitudes of whiskers were computed from the original (raw) whisker position traces. We set a 200 ms sliding window around each time point. Within this window, the "set point" was defined as the minimum (the most retracted) value while the "amplitude" was considered to be the difference between the maximum (the most protracted) and the minimum values. To remove the effect of abrupt changes -- because animals can begin a protraction or retraction in the 200 ms time window – we further smoothed the set point and amplitude traces, by applying another 200 ms sliding-window averaging. The "ΔR-L" values were computed by subtracting the left whisker value from its right counterpart on each time point. Thus the ΔR-L value ranged from -1 to 1: a value of "1" meant that the right whisker protracted maximally while the left whisker retracted maximally (i.e. the whiskers orient fully leftward). A value of "0" indicated that the left and the right whiskers protract/retract to the same extent. In

order to test asymmetry, a Wilcoxon's signed-rank test was used to test for the left vs. right normalized positions.

The nose position was normalized in the same manner as whisker position, with the exception that for the nose, the right-most and left-most positions become -1 and 1, respectively.

Normalization of duration for each behavioral state was performed by resampling. For each epoch, we fitted the (normalized) time base from -0.2 to 1.2 with steps being 0.01, where the epoch starts at time 0 and ends at time 1. The data points were resampled from the original time base (i.e. frames) to the normalized time base, using interpolation. The normalized data could then be used for calculating averages and standard error of mean (SEM) of whisker position in the course of an epoch. The normalized data could then be used for calculating averages and standard error of mean (SEM) of whisker position in the course of an epoch. We used a Wilcoxon signed rank test to assess whether whiskers were asymmetrically positioned in different behavioral states. We computed the R^2 values, and used the Kruskal–Wallis test to assess whether the position of whiskers on each side of the face, or the asymmetry of whisker position best captured the variance in side to side movement of the nose.

Results

A single trial in the Air-track plus maze

Behavior in the maze was self-initiated. There were no time constraints on when a trial began, or how long the animal took to perform a trial. The task was simple (**Figure 1C, Videos 1, 2**). At the beginning of a trial, the LED was turned on, mice were at the end of a lane, and had to move backward out of the lane to reach the center of the maze. When they reached the center of the maze, they turned it around themselves to find a dark lane, a lane in which the LED light was off. Mice then entered this lane – moved forward in it, all the way to end, to the opening in the lane where they waited for the reward tube to descend down toward them and then they licked the tube for the milk reward. When mice entered incorrect lanes, i.e. the lane where the LED light was on, they obtained no reward and had to move out of the incorrect lane, rotate the maze, and find the dark lane (**Videos 1, 2**). Over the course of training, mice learned to steer the maze and to select the correct lane quickly in each trial. During navigation, the behavior of mice could be divided into distinct states, consistent enough to be classified into epochs (**Figure 1C**): (1) the "Standing still" epoch that marked the beginning of a new trial, where the mouse stood still at the end of a lane, typically after previous reward delivery; (2) the "Backward" epoch, where the animal moved backward out of the lane to find another reward; (3) the "Turn" epoch, where the animal entered the center of the maze, and rotated the maze left or right around until it chose a new lane; (4) the "Forward" epoch, where the mouse moved forward into the new lane; (5) the "Expect reward" epoch, when the mouse waited at the end of a lane for the reward tube to descend; (6) if the chosen lane was correct, the reward tube descended towards the animal, and the "Lick" epoch began until the tube was retracted up and away from the animal.

Stereotypical whisking in each behavioral epoch

We tracked the movement of C1 and Gamma whiskers as mice (n = 5 animals) navigated the plus maze (**Video 1-3**). Whiskers on one side of the face in general moved to the same extent, and they were at similar set points with respect to each other in all behavioral epochs over the course of a trial. Therefore, for the rest of our analysis, we compare only the motion of a single whisker the rostral C1 whisker that was painted red, on each side of the face (**Figure 1B**).

In general whisker motion showed four stereotypical characteristics in different behavioral stages: 1) When mice moved in the maze, large amplitude (high frequency) whisking with a high degree of asymmetry between the sides of the face was evident (i.e. in backward, forward, or turning epochs) (**Figure 1C**, **2**). 2) In contrast, when mice were simply standing still in the maze, whisker movement was negligible and the set point of whiskers was retracted in this epoch (Standing still, **Figure 1C**, **2**). 3) When mice were standing still but were licking the reward tube, whisking was regular, and occurred at lower frequency than when mice were moving. When mice were standing still, but were licking, the set-point for whiskers was also protracted compared to times when mice were at the end of lane and standing still (**Figure 2**). 4) Finally, during reward expectation, whiskers were protracted and showed high amplitude, and high-frequency whisking.

Whisking was so stereotyped, that it served as a "signature" of each behavioral epoch (**Figure 2**). Even though the duration of a behavior, i.e. standing still or moving backward varied from trial to trial, on every trial the positioning of whiskers bilaterally reflected the behavior.

The whisker position traces from each trial were averaged after normalizing for time and bilateral extent of whisker movement (in order to compare whisker position on the two sides of the face) (**Figure 2B**). Averaging smoothed out the rhythmic whisking motion i.e. averaging removed the fast components of whisking. These averages confirmed what was evident in the raw data. *Each*

behavioral epoch thus had its own signature whisker position and this was independent of animals, sessions and trials, i.e. every trial in all animals had the same stereotypy.

Bilateral asymmetry signals turn direction

Mice had a strong rotation preference; they rotated the maze left or right, and rarely turned the maze ambidextrously in both directions. The mouse's decision to turn right or left was reflected in the asymmetric positioning of whiskers throughout a trial, which was evident very early during the backward movement epochs (n= 284 (224 left turning epochs + 60 right turning)). The asymmetry increased as the animal reached the center of the maze. In all trials, when mice reached the center of the maze, animals that turned left retracted their whiskers on the left side to their full extent while simultaneously protracting whiskers on the right side (**Figure 2A, B** top panels & **Video 1-2**). In contrast, animals that turned right retracted their right whiskers, and protracted their left ones, as they reached the center of the maze (**Figure 2A** bottom panels). Whiskers on the side of the face that were protracted, showed high amplitude whisking motion, while whiskers on the other side of the face retracted but displayed almost no rhythmic whisking motion (**Figure 2** and **Video 1-2**). The asymmetry that began at the onset of backward movement, increased while the animal was approaching the center of the maze and flipped during the turn (**Figure 2**). There was an inversion in the whisker position as the animals moved forward into a lane: whiskers that were completely protracted during backward movement were retracted, and vice versa. This inversion in whisker position was stereotypic, occurring automatically in every turn epoch (n= 289 (228 left turning epochs + 61 right turning)). Note that mice maintained this asymmetric position as they moved forward into the lane, primarily as a result of whisker contact with the wall on one side of their face. The

asymmetry gradually diminished as mice moved further forward into the lane (n= 286 forward (226 left + 60 right)).

To quantify these changes in asymmetry in the course of each behavioral epoch, we divided each behavioral epoch in two and compared the normalized left whisker position to the right whisker position (ΔR–L) at the beginning and end of each behavioral epoch. In all behavioral epochs, in both right and left turning animals, there was significant whisker asymmetry ($p < 0.05$, Wilcoxon sign rank test, n =139 to 228 epochs for left turning animals, and n = 40 to 61 epochs for right turning animals, **Figure 2C,**) and asymmetric positioning changed significantly ($p < 0.01$, Wilcoxon sign rank test) when whisker asymmetry in the first and second half of each behavioral epoch were compared (**Figure 2C,** data for right and left turning animals are binned together). There is a caveat to note here: the small difference in side to side positioning of whiskers during reward expectation, and licking arose in part from the direction of descent of the lick tube (**Videos 1, 2**). Independently of whether mice propelled the maze right or left, the reward tube descended toward the left side of their face, consequently, during licking and reward expectation the side to side asymmetry in all animals shows a similar pattern (compare the whisker position in reward expectation and licking epochs for the left and right turning mice in **Figure 2B** during reward expectation and lick epochs). But note that by the time the animal finished licking the reward tube, and decided to move backward in the lane, the lick tube related asymmetry was no longer evident; instead a small but consistent and significant ($p < 0.05$, Wilcoxon sign rank test) difference in side to side to position of whiskers became evident (compare the left turning and right turning data, in standing still epochs in **Figure 2A, B**). Taken together our results suggest that whisker asymmetry is a constant feature, and whiskers are actively repositioned as mice move through the maze. Whisker position at the beginning of a trial can predict decisions

mice make in imposing a movement direction on the maze, and the extent and direction of the asymmetry can effectively map the position of the animal in the maze.

In principle, whisker position asymmetry could arise from changes in amplitude of whisking or changes in set point (**Figure 3**). To examine whether amplitude or set point, or both contribute, the amplitude defined as the difference between the maximum and minimum whisker position, and the set point defined as the minimum, i.e. the most retracted position (**red trace, Figure 3A**) were measured (see materials and methods for details). Overall, the set point and the amplitude of movement both reflected asymmetry i.e. the protracted whisker position was related to both a protracted set point and larger amplitude movement on one side of the face. But in the course of the backward movement (n= 224 left turning epochs) and the turn (n=228 left), the set point and amplitude traces showed distinct patterns (**Figure 3B**, top panels for set point and bottom panels for amplitude in left turning animals), suggesting that these aspects of whisking change independently in the course of the animal's movement in the maze. When mice move backward the asymmetry in set point continued to increase (compare blue traces to red, **Figure 3B,** Left panel) i.e. mice protracted one side more. While amplitude was also asymmetric, the amplitude of whisking on the two sides of the face showed different dynamics than the setpoint. When mice turn, the changes in set point and amplitude occurred at different times (**Figure 3B**). When mice move forward (n= 226 left turning epochs) into a lane both the set point and amplitude of whisking change at the same time and in the same direction (**Figure 3B, Right panel**)

Sensory input and asymmetry

Whiskers are the primary tactile organs of rodents, and whisker asymmetry could arise from tactile input to the whiskers, especially in the Air-Track system where whisker contact with the walls

is a constant factor. To examine whether tactile input from whiskers drives the side to side asymmetry, we trimmed all whiskers off bilaterally and painted the remaining whisker stubs (**Figure 4A, right**). Mice were able to perform the task without their whiskers (**Figure 4B**). Furthermore, in these mice, whisker asymmetry still predicted the direction that the animal would move in, and whisker position still varied in a behavioral state dependent manner. There were still significant differences in direction of asymmetry during backward motion (n= 102 left turning epochs), turning (n= 101 epochs) and forward motion (n= 102 left turning epochs) ($p < 0.01$ Wilcoxon Sign rank test n = 101, **Figure 4C**). Note that trimming abolished side to side asymmetry during standing still, licking, and expecting reward. These effects of trimming during licking and reward expectation are in part related to the positioning of the lick tube as it descended towards the left side of the animal. That trimming abolished asymmetry during standing still epoch is likely related to the minimal initial asymmetry in this epoch, and the fact that the whiskers themselves were much shorter after trimming. These results rule out the influence of sensory input as the principal cause of stereotypical whisker positioning.

Whisker asymmetry in freely moving animals

Earlier work has shown that whisker asymmetry arises and is related to movement of the head (Towal and Hartmann, 2006; Grant et al., 2009; Schroeder and Ritt 2016). These earlier studies were all in freely moving animals. To examine whether the results we obtained in head-fixed mice were an artifact of head fixation, we tracked whisker motion in 2 freely moving animals (**Figure 5**). These animals had been previously trained on the plus maze while they were head-fixed. Whisker tracking in freely moving animals was constrained to just the central portion of the maze, i.e. just as the mouse backed out of a lane, turned and entered another lane. In freely moving mice, head

movement made tracking of whisker position difficult. Not only did the head move up and down, mice often rotated their head from side to side. Nevertheless, in a limited set of observations, when accounting for head angle, whisker position was asymmetric in the same direction, at the same places, in freely moving animal as in the head fixed mouse (**Figure 5**). Whiskers on the animals left side were retracted, right side protracted as the mouse exited the lane (**Figure 5**, top panels, backward) and began to turn left (**Figure 5**, bottom panels, turn). Once the mouse was in the center of the maze and turning, the whisker position flipped and the mouse protracted whiskers on the right side and retracted them on the left. The white insets in this figure show the position of whiskers for head fixed mice when they were in a similar position in the maze. Head fixed mice showed similar asymmetry to that seen in the freely moving mice.

Nose movement: relationship to whisker asymmetry

The facial muscles that move whisker pad and whiskers, are controlled by a central pattern generator, that controls breathing and sniffing (Moore et al., 2013; Moore et al., 2014), some of these muscles also control the motion of the nose (Haidarlu et al., 2012; Haidarlu et al., 2015); in fact the nose and whiskers also move in a coordinated fashion (McElvain et al., 2018). Here we examined whether the nose moved in a behavioral state-dependent manner, and whether it was correlated to whisker motion. As the maximum intensity projection of nose movement over 30 trials shows the nose moved a fair amount in the course of Air-track behaviors (**Figure 1B**) and moved differently in each behavioral epoch (**Figure 6A –** bottom trace). Side to side nose movement (i.e. left - right) was best related to the asymmetry of whisker position on the two sides of the face (**Figure 6A**, middle trace), it was not as nicely related to the motion of whiskers on either side of the face (**Figure 6A**, top

traces, $p < 0.001$ Kurskal-Wallis test). This becomes especially evident when whisker asymmetry and nose movement were plotted together: bilateral whisker movement (Δposition = R-L, purple) and nose movement (green) changed together (**Figure 6B**). Note that the end of the *Turn* epoch and the beginning of the *Forward* epoch were uniformly associated with whisker contact to the wall which was likely to have interfered with the relation between whisker asymmetry and nose movement. These data show that just as whisker asymmetry is stereotyped in the behavioral epochs, nose movement was also stereotypical.

Discussion

This work shows that whisker motion is not just about tactile behaviors, or even about exploring the environment: instead the position of whiskers, may be related to motor preparation and action. For an observer the positioning of whiskers also serves as a map of the mouse's location in a maze. Each behavioral epoch has signature features that correlate with the movement and positioning of the whiskers (and the nose). While whisking frequency and set point varied with the behavioral epochs, the side to side position of whiskers was most clearly related to the behavioral state (**Figure 7).** For example, motion by the mouse forward or backward was associated with the onset of whisking; licking was associated with a protracted set point for whiskers (compared to the set point when mice were simply standing still), but asymmetry in side to side whisker position alone was a key indicator of the animal's position in the maze. Even when mice simply moved backward in a straight line, the extent and direction of asymmetry correlated with the turn that the animal was about to initiate.

A priori we might expect that a portion of the whisker asymmetry arises from tactile input from the whiskers. After all each whisker is associated with thousands of neurons in the rodent somatosensory cortex (Van der Loos and Woolsey, 1973; Oberlaender et al., 2012), and stimuli to individual whiskers evoke a cortical response (Armstrong-James et al., 1992; Simons, 1985; Petersen et al., 2003; Hasenstaub et al., 2007). Furthermore, whiskers are known as tactile elements, used by rodents in social settings (Lenschow et al., Neuron 2015), for detecting the location and presence of objects (Hutson and Masterton, 1986), for discriminating between fine textures (Carvell and Simons,1995; Chen et al., 2013; Kerekes et al., 2017;), and used as an aide for guiding mice as they navigate their environment (Hutson and Masterton, 1986; Carvell and Simons, 1990; Brecht et al.,

1997; Voights et al., 2015). In the plus maze used here the floor, and walls of the maze, their texture, the edges of the lane openings can all serve as tactile input. Whiskers contact the wall as the animal navigates the maze, and this contact might result in the asymmetry. But our work shows that while whiskers do contact the walls, contact isn't the main reason for side to side asymmetry; the side to side asymmetry persists even after whiskers have been trimmed to stubs that provide little tactile input. This suggests that the asymmetry arises as a postural adjustment or as a preparation for the actions that can follow hundreds of milliseconds after the initial asymmetric positioning of whiskers. That the level of asymmetry increases and decreases in a behaviorally relevant fashion – i.e. it increases as the animal approaches a turn, and the protracted and retracted side flip as the animal makes the turn -- suggest that this asymmetry is related to postural adjustments or active positioning of the whiskers (**Figure 7**). The postural adjustments – moving whiskers, positioning them asymmetrically -- that the animal makes before moving correlate with the rotation the mouse will impose on the maze and signal the animals intention to go, to move, and to prepare for a turn. A priori, it is possible that motor preparation involves changes in breathing triggered by anticipation of the additional effort that walking involves, and this change in breathing triggers whisking that co-occurs with each bout of movement the animal makes. But asymmetry in whisker set point probably does not arise from breathing or sniffing which are fast events and occur simultaneously in both nares. Whisker asymmetry is likely to be an anticipatory postural adjustment, reflecting the motor plan that mice develop in advance of the turn that they intend to impose on the maze.

A key implication of this work is that on a trial by trial basis, mice signal what they are doing, where they are, by activating brain circuits that guide whisker movement and movement of the nose. In every single trial, mice make the same stereotyped whisker movements when they reach the

center of the maze to look of the correct lane to enter. The circuits signaling whether to go forward or backward or turn, must be activated at the same time that the circuits related to whisking, whisker set point, whisking asymmetry, and nose position are activated. In sum, our work suggests that it is possible to discern the internal behavioral state of the animal from the whisker position (**Figure 7**). Thus, brain circuits that control the animal's motion, whisking and motion of the nose are engaged differentially during each behavioral epoch. Nose and whisker position could reflect attentional state of the animal, with the set point and whisking amplitude independently reflecting another as yet unknown, internal brain state variable.

The degree of correlation between whisker position and the animals behavioral state – its preparatory state -- is surprising. That the internal state of the animal is related to, even predicted by the positioning of, and motion of these ostensibly tactile, sensory organs is remarkable.

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Figures and Legends

Figure 1. *A trial in a "Air-track" plus maze with whisker tracking.* **A.** *Mice in a plus maze.* A schematic of the air track plus maze used for this study is shown here with a head fixed mouse, navigating the maze. Mice were trained to rotate the maze away from a lane that had a LED light and kept rotating the maze around themselves to find the dark lane, which they entered, and traversed to the end, in order to obtain a milk reward**.** Note that the mouse, the LED, and the location of the reward remained fixed in place, all that moved was the maze. **B.** *Maximum intensity projection of a mouse, its painted whiskers and nose*. **Left.** The painted whiskers on each side of the face, and a spot painted on the nose are shown here in a single frame. Some of the adjacent, un-trimmed whiskers are clearly visible here. **Right.** Maximum intensity projection of 30 trials. The movement of the whiskers around the face of the mouse forms a halo composed of the two colors painted on the whiskers and reflects all the positions the two painted whiskers occupied in the course of 30 trials in the maze. Furthermore, the small spot painted on the nose transforms into a large spot over the course of the thirty trials, indicating that the nose moves a lot as the animal traverses the maze. **C.** *A single decomposed trial of behavior and bilateral whisker motion***.** We tracked the motion of two whiskers (rostral one painted red, caudal one painted green) bilaterally for the duration of each trial. Once a trial ended, the lick tube was retracted, LED light turned on indicating that the mouse was in the wrong lane. At this point, mice often waited at the end of the lane without moving much (standing still– dark grey bar on top) for seconds to minutes, before they exited the lane by going backward (orange). Then the mouse turned and rotated the maze around itself (yellow), until the LED at the end turned off. Mice had to enter the dark lane, move forward into it (dark blue). At the end of the lane they waited for lick tube to descend (expect reward – light grey), and lick for the reward

(purple). There was a delay between the end of the forward motion, and the time for the lick tube to descend to the animal – reward expectation. Two whiskers on each side of the face – one painted red (rostral whisker), and the other painted green (caudal whisker) -- could be tracked for the extent of the trial. The position of the whiskers over the course of the trial was related to the behavioral epochs. Note that whisker motion was related to the animal's motion. Whisking was apparent when the animal was moving backward or rotating the maze or going forward. In contrast when the animal was standing still whiskers did not move much. And when the animal was licking the reward, the pattern of whisker motion was distinct – low amplitude, rhythmic, bilaterally symmetric -- compared to epochs where the mouse was moving the maze.

Figure 2. *Patterns of whisker motion during different behavioral epochs.* **A.** *Normalized whisker position in single trials and behavioral epochs*. The position of whiskers on each side of the face (red is right side, blue is the left side) in the course of each behavioral epoch (without averaging or smoothing), taken on different days are shown from 3 ***left*** turning animals (top, above the dashed line), and 2 ***right*** turning animals (below the hashed line). A trial was decomposed into behavioral epochs selected for analysis. **B**. *Normalized average whisker position in different behavioral epochs.* Whisker asymmetry in different behavioral epochs averaged during behavior for left turning (top), and right turning (bottom) mice. Normalized whisker position on left and right side, for left-turning (n=3) and right-turning (n=2) animals. While whiskers were retracted almost equally on both sides of the face, before the trial began the average normalized data reveal that as mice begin to go backward whisker positioning becomes asymmetric and the asymmetry was related to the direction of turn mice impose on the maze. When mice moved the maze to the right (bottom panels), the positioning

of whiskers was asymmetric, and a mirror image of how whiskers were positioned when mice turned the platform to the left (top panels). The blurred red and blue traces in the background show whisker positions in every single trial. The epochs used for analysis are defined by the grey bars behind the average whisker position traces. **C.** *Average whisker position in first and second half of behavioral epoch*. The average side to side position of whiskers changed significantly in all behavioral epochs (Wilcoxon sign rank test, $p < 0.05$ single asterisk and $p < 0.01$ double asterisk, combined for right and left turning trials). The normalized mean asymmetry for the first half of each epoch was 0.02 (SEM 0.01) for standing still, 0.23 (SEM 0.01) for backward, 0.56 (SEM 0.01) for turn, -0.2 (SEM 0.02) for forward, 0.023 (SEM 0.01) for expect reward, 0.12 (SEM 0.01) for lick. The normalized mean asymmetry for the second half of each epoch was 0.02 (SEM 0.01) for standing still, 0.432 (SEM0.01) for backward, for -0.19 (SEM 0.01) turn, 0.06 (SEM 0.02) for forward, 0.1 (SEM 0.01) for expect reward, and 0.04 (SEM 0.06) for lick. In the right turning animals (data is not shown), the mean values were -0.65 (SEM 0.02) for standing still, -0.14 (SEM 0.02) backward, -0.46 (SEM 0.01) for turn, 0.08 (SEM 0.02) forward, 0.08 (0.02) expect reward, and 0.27 (0.03) lick in the first half and -0.09 (SEM 0.02) stand still, -0.31 (0.02) backward, 0.119 (0.02) turn, -0.03 (0.03) forward , 0.28 (0.03) expect reward, and 0.26 (0.04) lick. The light red and blue traces show the single trial data that was used to compute the average whisker positions on two sides of the face. The epochs used for analysis are defined by the grey bar.

Figure 3. *Asymmetry of set point and the amplitude.* **A**. *Single example illustrating the extraction of the set point and the amplitude of whisking.* A 200 ms-sliding window was applied to the whisking dynamics (Raw, gray) to detect the local minima/maxima. The minima were defined as the set point (magenta), whereas the differences between the maxima and the minima were defined as the

amplitude of whisking (Green). Note that the values were further smoothed using a 200 ms sliding-window to remove components that occur due to abrupt changes in whisker motion. **B**. *Behavioral state-dependence of the set point (top) and the amplitude (bottom).* When mice were moving the maze backward, and or turning, the asymmetry in set points and asymmetry in amplitude of whisker motion showed different temporal features (compare top and bottom plots). The normalized mean asymmetry for the *set point* of whisker position in the first half of each epoch was 0.17 (SEM 0.006) for backward, 0.47 (SEM 0.007) for turn, -0.13 (SEM 0.01) for forward motion. The normalized mean asymmetry for the set point in the second half of each epoch was 0.31 (SEM 0.007) for backward, for -0.03 (SEM 0.006) turn, 0.05 (SEM 0.01) for forward motion. The normalized mean asymmetry for the *amplitude* of whisking in the first half of each epoch was 0.12 (SEM 0.008) for backward, 0.01 (SEM 0.007) for turn, -0.12 (SEM 0.02) for forward motion. The normalized mean asymmetry for the *amplitude* of whisking for second half of each epoch was 0.22 (SEM 0.007) for backward, for -0.24 (SEM 0.006) turn, -0.035 (SEM 0.01) for forward motion. The light red and blue traces show the single trial data that was used to compute the average whisker positions on two sides of the face. The epochs used for analysis are defined by the grey bars in the background. Data shown here is from left-turning animals.

Figure 4. *Whisker asymmetry was not related to tactile input*. **A.** *Single frames of whisker position in normal (left) and trimmed (right panel).* In left turning mice when whiskers were trimmed, the whisker stubs could still be tracked, side to side asymmetry of whisker position was still evident. **B**. *Normalized average whisker position after trimming in different behavioral epochs.* The asymmetric positioning of whiskers persisted and was evident when mice began to move backward, and persisted

through the trial, as mice moved further backward and made left turn. **C**. *Mean asymmetry after trimming*. Even after trimming, there was significant asymmetry (Wilcoxon Sign Rank test, $p < 0.01$, based on n= 102 for backward motion, n=101 turn, n = 102 forward epochs). The normalized mean asymmetry of whisker position in the first half of each epoch was 0.28 (SEM 0.02) for backward, 0.44 (SEM 0.013) for turn, 0.06 (SEM 0.02) for forward motion. The normalized mean asymmetry in the second half of each epoch was 0.48 (SEM 0.01) for backward, for -0.1 (SEM 0.02) turn, 0.06 (SEM 0.01) for forward motion. The light red and blue traces show the single trial data that was used to compute the average whisker positions on two sides of the face. The epochs used for analysis are defined by the grey bars in the background of the whisker position traces.

Figure 5. *Asymmetry in freely moving animals.* Mice that had previously been trained in the Air-Track were imaged navigating the maze without head-fixation. While whisker asymmetry persisted in freely moving animals and looked qualitatively similar to that seen in head fixed mice, it was also associated with changes in head position. The head and whiskers have been outlined here to make it easier to see whisker position and the animals head rotation. The direction that the animal was moving in is given by the arrows. In freely moving animals, head rotation, and angle relative to body also played a role, but the side to side asymmetry was still pronounced and similar to that seen in animals that were head fixed. The white schematic of the mouse (two right panels) shows the position of whiskers in a head-fixed animal at the same location in maze.

Figure 6. *Whisker asymmetry and nose movement*. **A***. The nose moves when mice move*. Whenever the mouse moved its whiskers (top traces red and blue), whisker movement was asymmetric (middle trace) and the mouse moved its nose (third trace from top). The right-left movement of the nose was

significantly and best correlated with the complex motion of whiskers (second trace) on both sides of the face (Kurskal Wallis test $p < 0.001$), not with motion of whiskers on any side of the face. **B.** *Average nose movement related to whisker asymmetry in the different behavioral epochs*. When mice are standing still, licking or expecting reward the nose (green trace) does not move and asymmetry in whisker position (purple trace) is minimal but when mice are moving the maze, when mice go backward, turn and go forward the nose also moves and this movement is related to the asymmetric positioning of the whiskers.

Figure 7. Whisking is a signature of motor plan and behavioral state. A. *Right and left turning mice, plan their movement in the plus maze, and this plan is reflected in how they position their whiskers*. Left turning mice protract their whiskers on the right side while moving backward, and right turning mice, protract their whiskers on the left side while moving backward (mice in the semicircles show whisker position for right and left turning mice). The numbers next to the mice indicate the different behavioral epochs. At the onset of backward movement (1) whiskers are already asymmetric. As the animal continues moving backward toward the middle of the maze (2) the asymmetry in whisker position increases until the mouse exits the lane and is in the middle of the maze (3). As the mouse moved forward into a new lane, the asymmetric whisker positioning flipped (4) and then as the mouse proceeded into the lane, the asymmetry diminished (5). The trial ends when the animal is licking (6). **B***. Schematic of whisker position as a signature of behavioral state and movement preparation*. Asymmetry in whisker position and whisker set point both reflect the distinct behavioral state of the animal. The numbers in the schematic are the behavioral states (related to the numbers used in A).

Videos

Video 1. A wide-angle view of a mouse performing a single trial in the maze. In this trial, the mouse backs out of a lane, and partially enters a lane where the LED light is still on, then backs out and enters a dark lane and gets rewarded for correct performance. The nose and two whiskers were painted bilaterally.

Video 2. A close up view of a mouse performing a single trial. In this trial, the mouse backs out of a lane, and enters an incorrect lane and waits there, then backs out and enters a dark lane and gets rewarded for correct performance.

Video 3. Video of the masks used for each whisker and nose in a single trial.

Figures

Figure 1

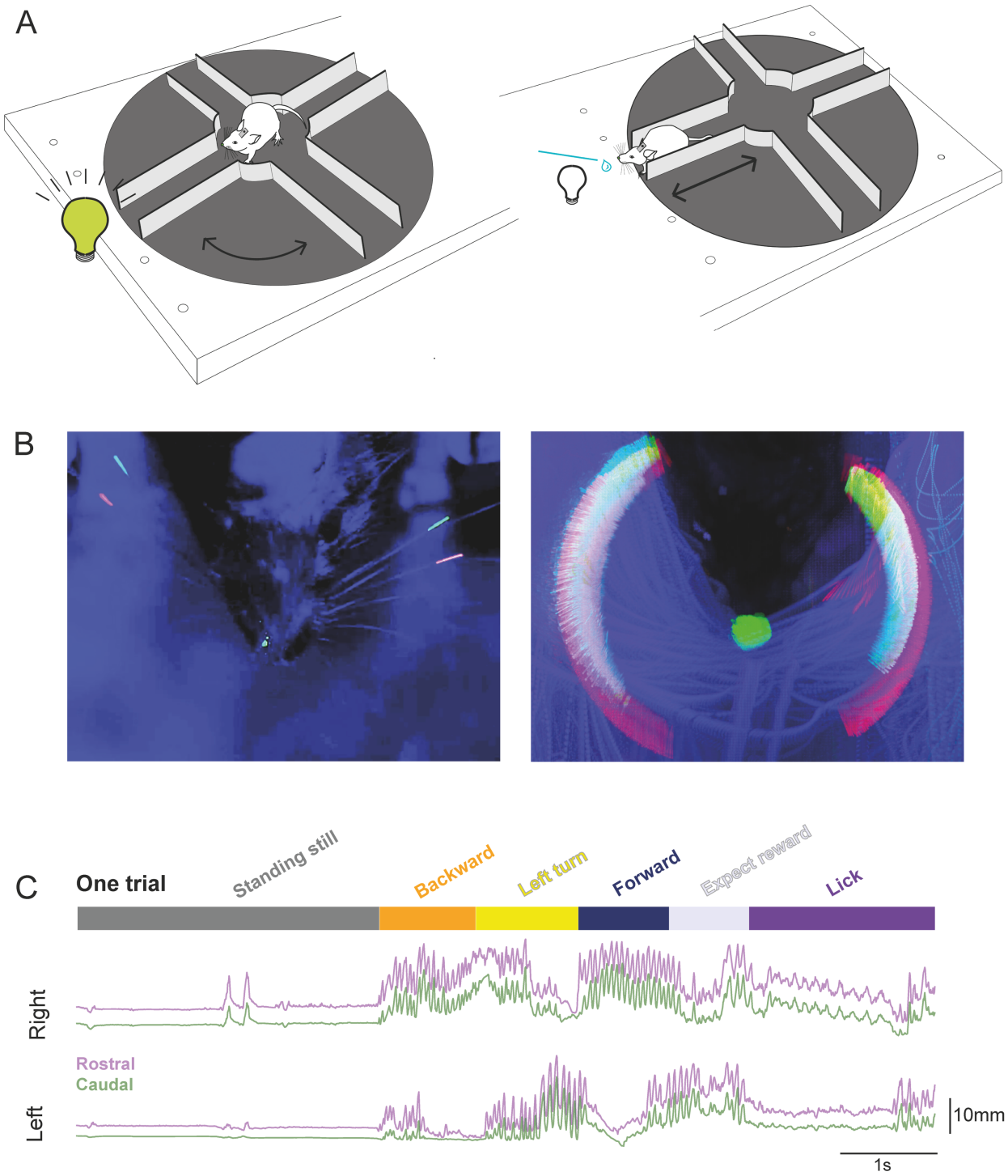


Figure 2

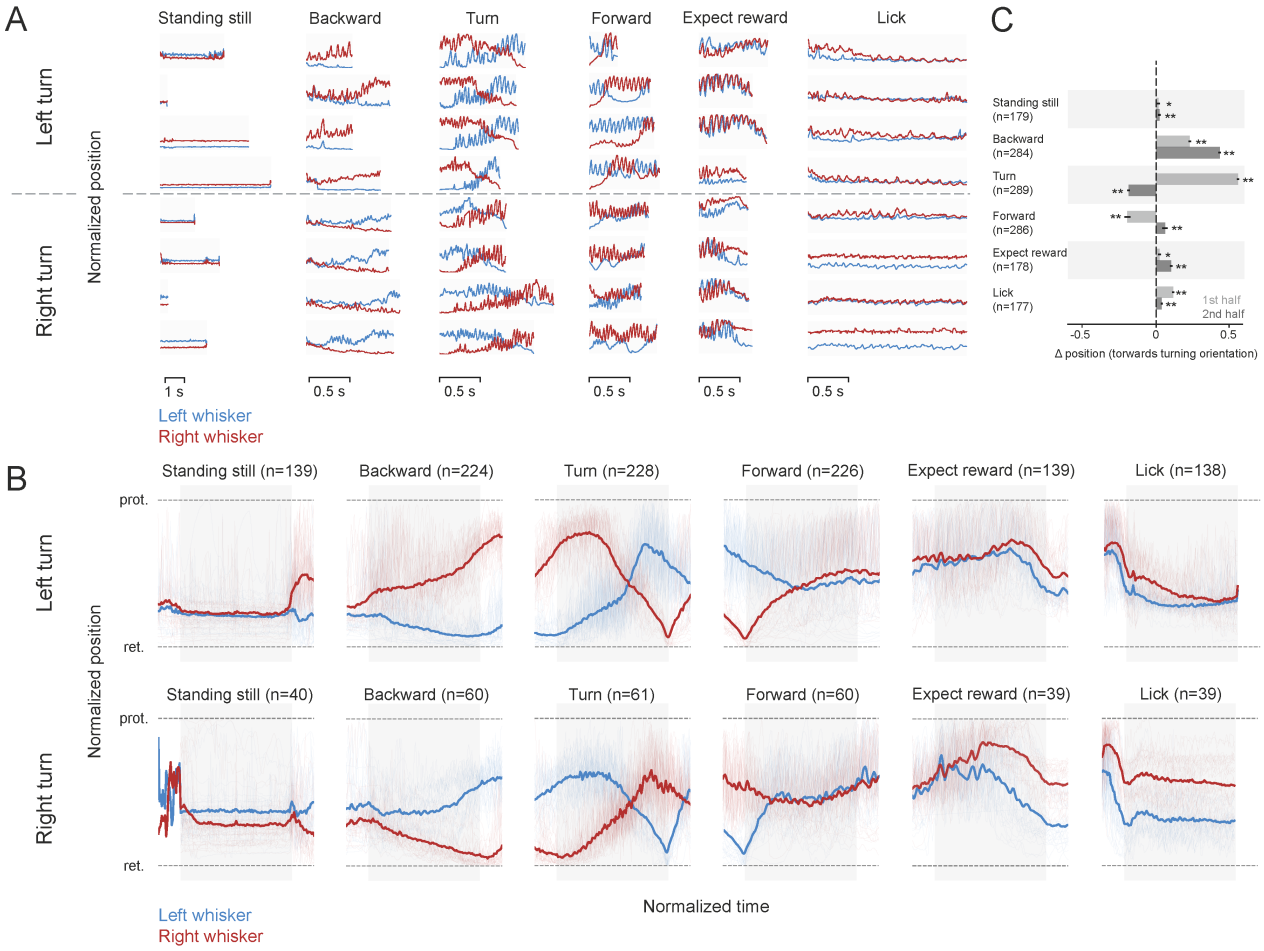

A
Standing still
Backward
Turn
Forward
Expect reward
Lick
Left turn
Right turn
Normalized position
1 s
0.5 s
Left whisker
Right whisker
C
Standing still (n=179)
Backward (n=284)
Turn (n=289)
Forward (n=286)
Expect reward (n=178)
Lick (n=177)
1st half
2nd half
-0.5
0
0.5
Δ position (towards turning orientation)
B
Standing still (n=139)
Backward (n=224)
Turn (n=228)
Forward (n=226)
Expect reward (n=139)
Lick (n=138)
Standing still (n=40)
Backward (n=60)
Turn (n=61)
Forward (n=60)
Expect reward (n=39)
Lick (n=39)
prot.
ret.
Normalized time

Figure 3

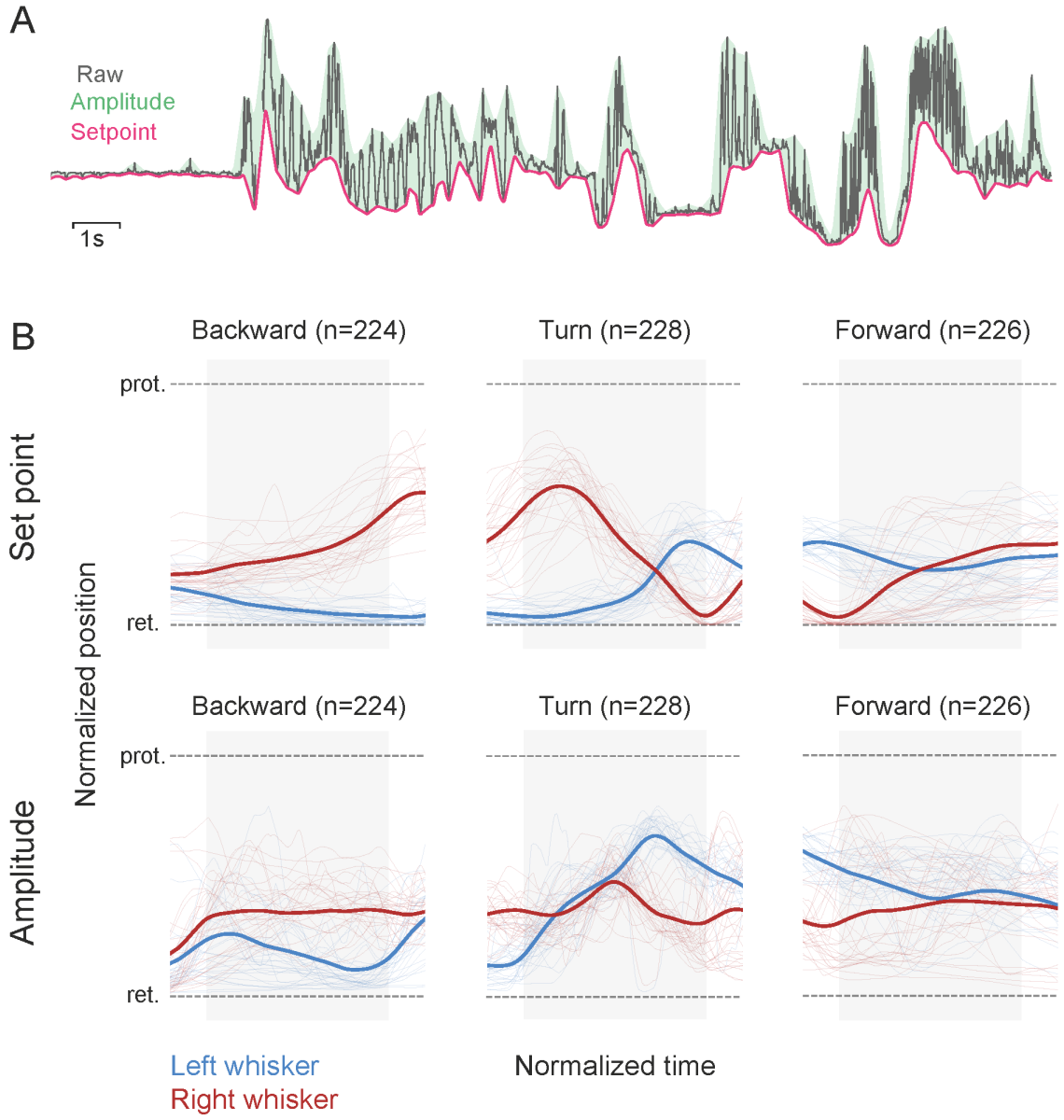

A
Raw
Amplitude
Setpoint
1s
B
Backward (n=224)
Turn (n=228)
Forward (n=226)
prot.
ret.
Set point
Normalized position
Amplitude
Left whisker
Right whisker
Normalized time

Figure 4

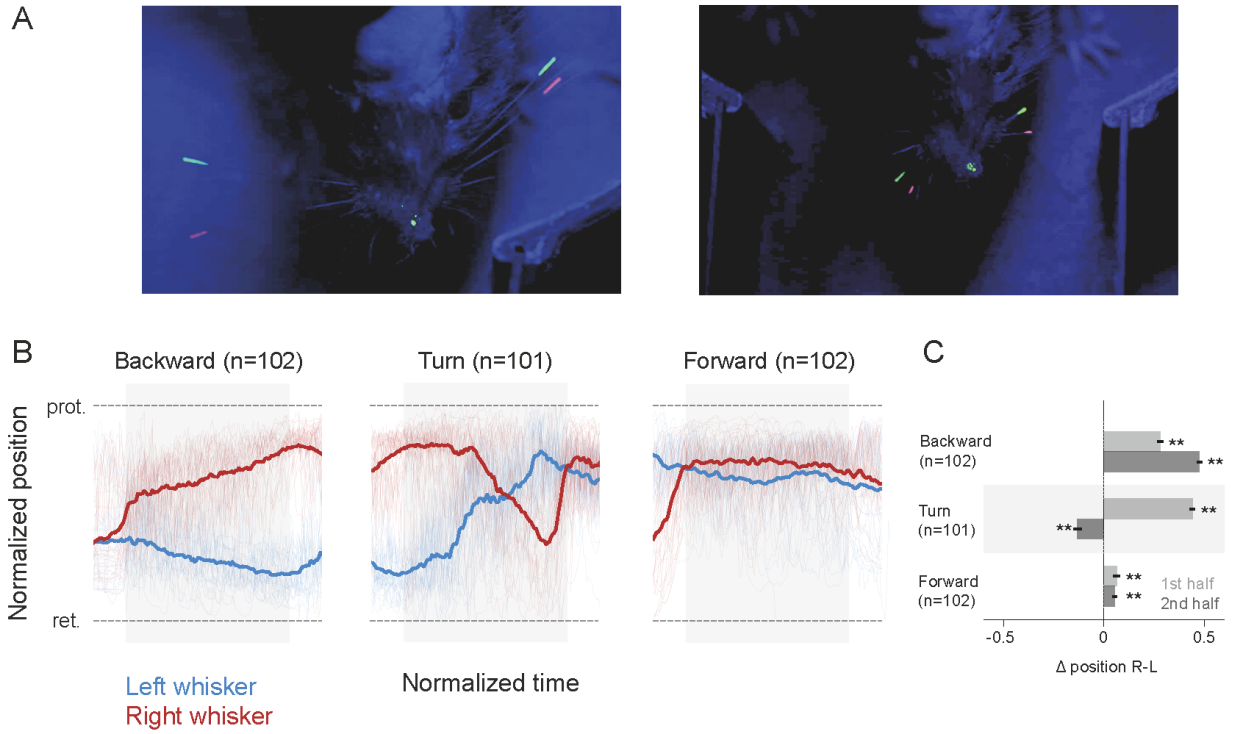

A
B
Backward (n=102)
Turn (n=101)
Forward (n=102)
Normalized position
prot.
ret.
Left whisker
Right whisker
Normalized time
C
Backward
(n=102)
Turn
(n=101)
Forward
(n=102)
1st half
2nd half
-0.5
0
0.5
Δ position R-L

Figure 5

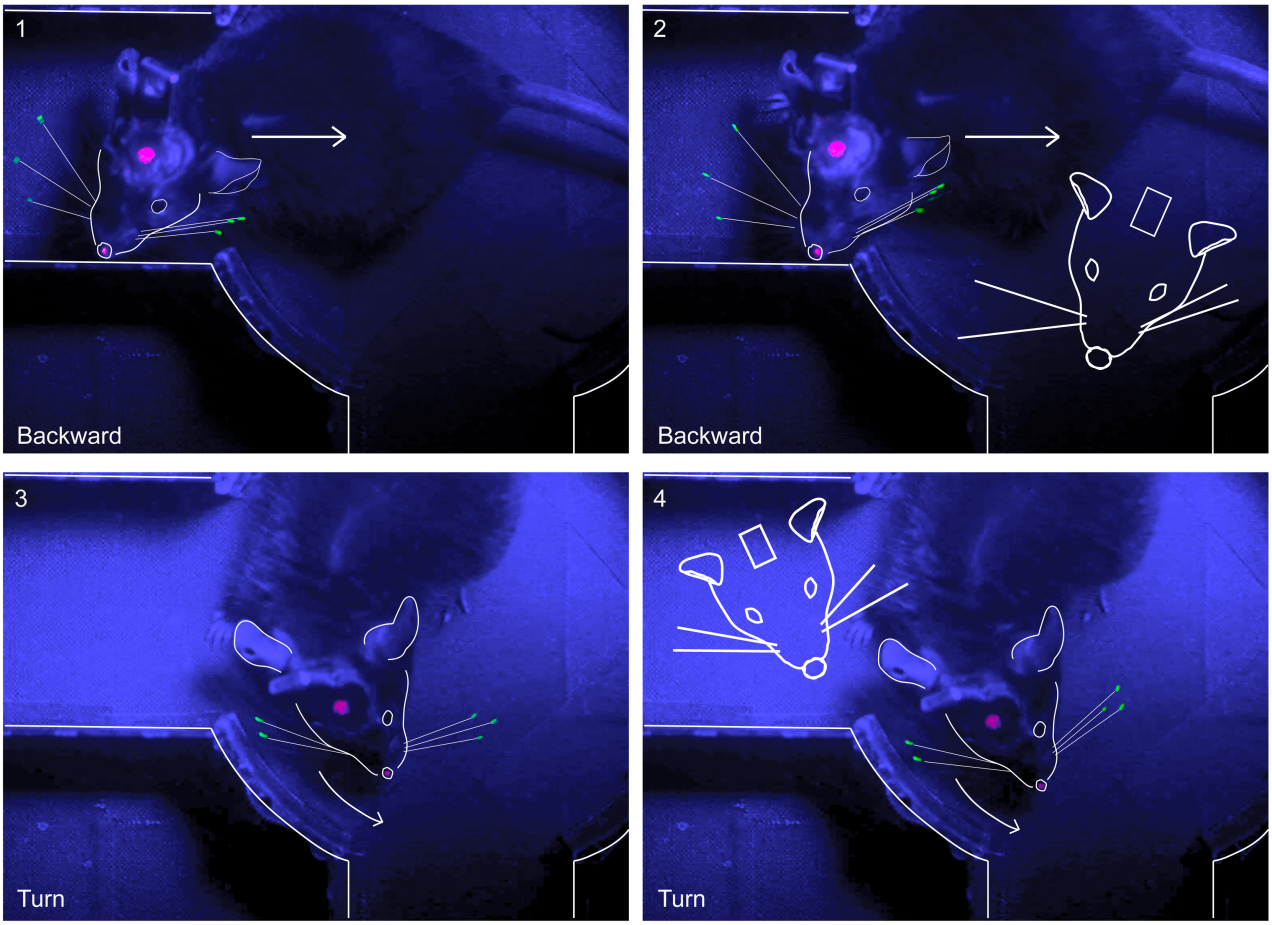


Figure 6

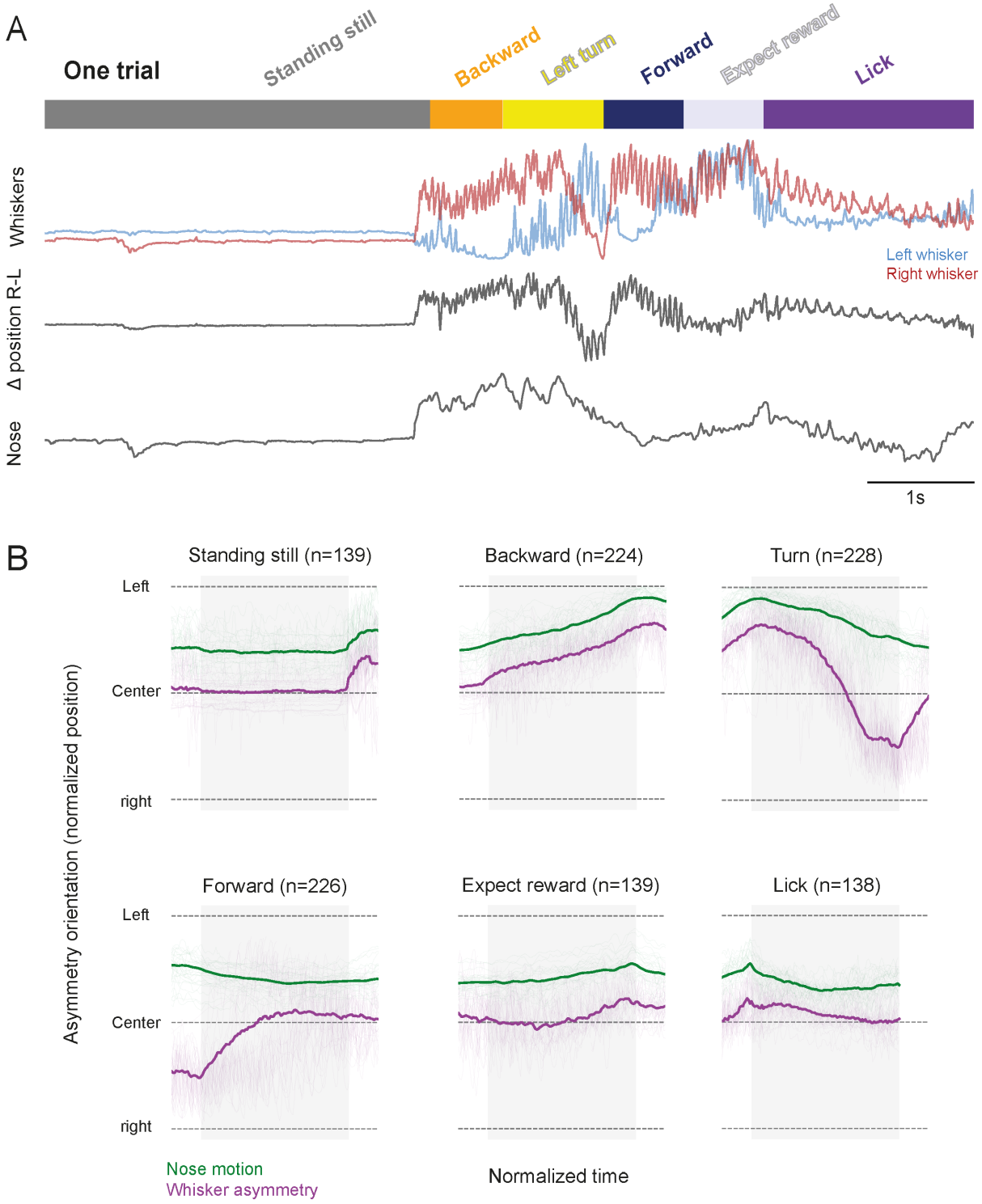
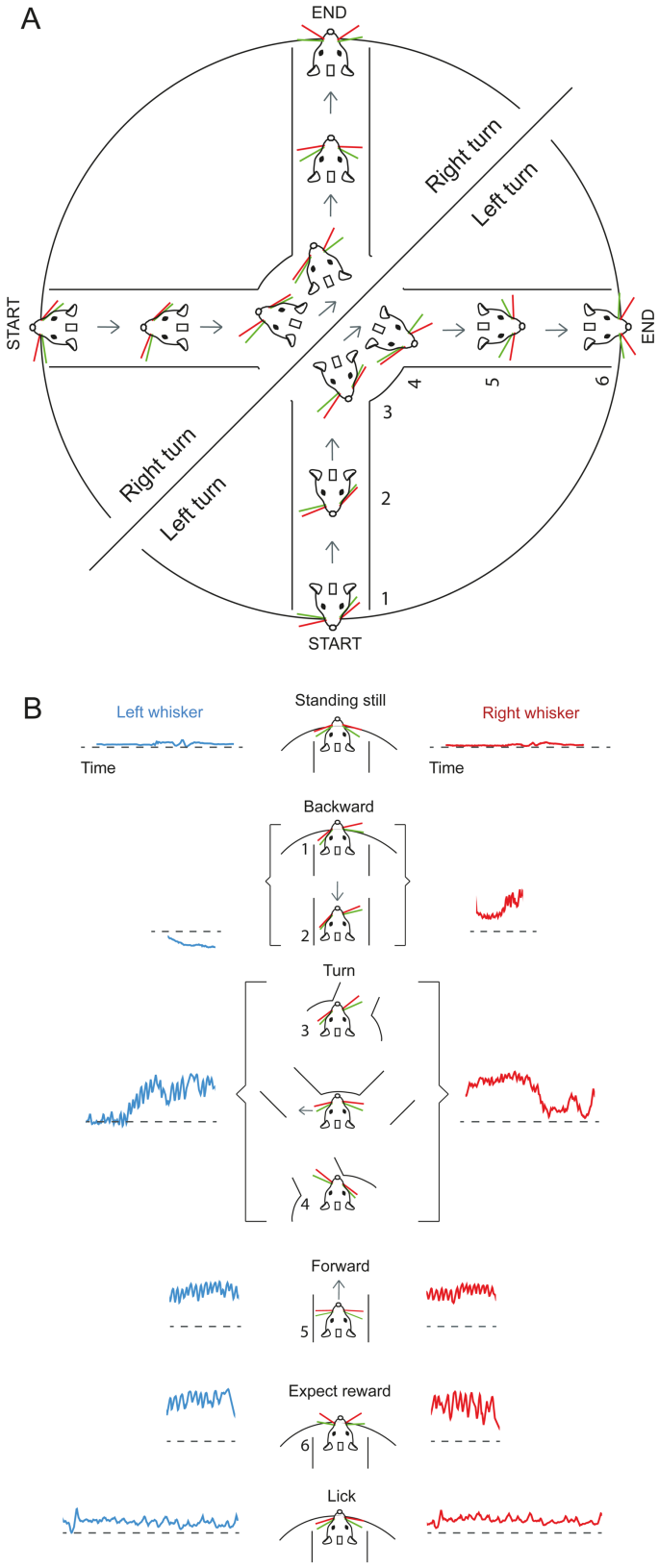

A
One trial
Standing still
Backward
Left turn
Forward
Expect reward
Lick
Whiskers
Δ position R-L
Nose
Left whisker
Right whisker
1s
B
Standing still (n=139)
Backward (n=224)
Turn (n=228)
Forward (n=226)
Expect reward (n=139)
Lick (n=138)
Left
Center
right
Asymmetry orientation (normalized position)
Nose motion
Whisker asymmetry
Normalized time

Figure 7


A
END
START
END
START
Right turn
Left turn
Right turn
Left turn
1
2
3
4
5
6
B
Left whisker
Time
Standing still
Right whisker
Time
Backward
1
2
Turn
3
4
Forward
5
Expect reward
6
Lick