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RESEARCH REPORT

# Long and Short Whiskers Help Guide and Regulate the Precision of Rat Orientation Behavior

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## ABSTRACT

Rat whiskers comprise an "active sensing" system involving two functional subdivisions: long whiskers for object localization and short whiskers for object recognition. To explore their respective roles in orientation, rats were trained in a reaching–grasping task. Specifically, four consecutive salient frames were identified in control rats: (i) whisker touch (Wt), the long whiskers came into contact with the front wall; (ii) first nose touch (Fnt), the rat brought the nose into contact with the wall; (iii) poke (Pk), the rat inserted its nose through the slot and placed short whiskers on the shelf, exploring it until the pellet was detected; and (iv) nose elevation (Nel), the rat raised its nose until reach start. These frames were used to subdivide orientation behavior into three specific phases: Wt–Fnt, Fnt–Pk, and Pk–Nel. To determine their respective roles in orientation, the rats performed the task after either long whiskers trimming or short whiskers shaving. Data evidenced a temporary loss of orientation followed by a recovery specific to each experimental group. Trimmed rats presented incomplete trials with loss of invariance, longer Fnt–Pk duration, and an increased number of nose touches. Shaved rats displayed longer trial duration and longer Pk–Nel interval. This duality is explainable by a consecutive use of the two kinds of whiskers and confirms their different roles in the multisensory integration necessary for each orientation phase. The data suggest that the long whiskers can be viewed as a spatial orientation system acting as a precision mechanism guiding head position in the context of coherent behavior.

## 1 | Introduction

For many years, studies have been addressing the role of the whisker/snout sensory system in the rat's interaction with the surrounding world. The whiskers are thought to act as a form of "active sensing" system (Mitchinson et al. 2011; Severson and O'Connor 2017) whose tactile sensory abilities provide signals for many basic functions, such as orientation, tracking objects/surfaces in space, gap crossing, locomotion, and social interaction (Ahissar and Knutsen 2008; Arkley et al. 2014; Wolfe et al. 2011; Diamond and Toso 2023). There are two functional subdivisions to the rat's whisker system: the long and the short whiskers. The long whiskers are considered a distance-detecting sense organ, serving to locate objects,

**Abbreviations:** Fnt, first nose touch; Fnt–Pk, first nose touch–poke; Nel, nose elevation; Pk, poke; Pk–Nel, poke–nose elevation; Wt, whisker touch; Wt–Fnt, whisker touch–first nose touch.

whereas the short whiskers are treated as a sense organ for object recognition (Brecht et al. 1997). In oral grasping, the long and short whiskers guide the snout–pellet interaction in different ways (Parmiani et al. 2023); in other functions, the role of the short whiskers is still unknown. Contact between the long whiskers and a surface often induces orientation toward that surface, which is then further explored by whisking, with a numerical increase in contacts within a sort of attentional zone (Mitchinson and Prescott 2013; Mitchinson 2015). At the same time, the tip of the snout/nose is guided by whisking toward the surface of interest and explores it through repetitive nose touches, i.e., nosing (Welker 1964). There is substantial evidence suggesting that nosing is part of a general orientation system operating synchronously with other rhythmic facial activities at almost 10 Hz, which is the mean characteristic frequency for exploratory behavior (Kurnikova et al. 2017; Ranade et al. 2013).

A suitable experimental model for studying how whisker/snout guide head/nose movements in a monitored context is the so-called "skilled reaching" task, in which a forelimb is extended to grasp food for eating. Skilled reaching is considered to be composed of three successive learned responses: (1) orientation, (2) transport, and (3) withdrawal (Whishaw and Pellis 1990; Whishaw 1996; Gharbawie and Whishaw 2006; Alaverdashvili et al. 2008; Alaverdashvili and Whishaw 2013). Orientation involves locating a reaching box's front wall, slot, and shelf through synchronized goal-directed positioning of the whiskers and head/nose. Transport involves a semiballistic forelimb movement across the slot to grasp the pellet. Finally, withdrawal consists of the paw being used to retrieve the pellet through the slot so it can be eaten. Inside a reaching box, the rat uses its whiskers and tactile nose sense, olfactory cues, and proprioception, to locate food and to define a reaching path for the paw to target the pellet (Welker 1964; Whishaw and Tomie 1989; Whishaw and Karl 2014; Sherman et al. 2017; Whishaw et al. 2017; Parmiani et al. 2018). Orientation and reaching are dissimilar in multisensory integration required and are differently modulated by environmental context (Towal and Hartmann 2006; Mitchinson et al. 2007; Sutter et al. 2014; Whishaw and Karl 2014; Parmiani et al. 2018).

In fact, the multisensory integration processes involved are assumed to adjust the contribution of each sense to a multisensory task moment by moment and do not take place when sensory inputs are spatially or temporally separated or suppressed (Calvert and Thesen 2004; Gepshtein and Kubovy 2005; Sutter et al. 2014). Equally, two stimuli from the same sensory modality can also interact with each other with a synergistic or consecutive effect (Inagaki et al. 2020; Parmiani et al. 2023). Otherwise, rats hold stimuli in their working memory and integrate separated sensory modalities to create a supramodal object representation (Raposo et al. 2012; Nikbakht et al. 2018). The rat's whiskers system is one of the most helpful and commonly employed models to investigate sensorimotor integration (Diamond and Toso 2023). Their use of long and short whiskers depends on the ecological or experimental context, i.e., on the environmental demands. For instance, rats exploring an object use the long and short whiskers simultaneously and/or consecutively (Hartmann 2001), whereas rats trained in oral grasping use their long or short whiskers consecutively, in relation to the specific orientation phase of the task itself (Parmiani et al. 2023).

Removing or damaging the long whiskers in lesion studies alters or disrupts many functions involved in various behaviors, presumably the consequence of the loss of the tactile signals they send (for a review, see Ahl 1986; Goldberger 1977; Diamond and Toso 2023). Rats in which long whiskers have been bilaterally damaged exhibit a change in nosing activity, exploring the surfaces of interest with repetitive nose touches (Symons and Tees 1990; Parmiani et al. 2018), whereas rats subjected to unilateral whisker removal present a short-term asymmetry in their explorative behavior. This asymmetry is greatest and most clearly evident during the first minute of testing, but it recovers in up to 3 days (Milani et al. 1989). Based on the above findings, we devised two complementary experiments to define the specific roles of the long and short whiskers in rats' control of orientation behavior during a reaching task. The first was to be conducted by a group of rats that had had their long whiskers trimmed (trimmed rats) and the second by another group whose short whiskers had been shaved (shaved rats). For comparison purposes, trials were recorded in the same rats before and after lesion. To define the specific role of long and short whiskers in orientation movement, we chose a micro behavior termed skilled reaching as a target task where long and short whiskers are sequentially involved during a single pellet task execution.

Though the short-term effects of surgery were followed by a rapid recovery, the resulting data evidenced that the long and short whiskers make different contributions to the various phases of orientation behavior. Moreover, the data suggest that the long whiskers act as a precise regulation mechanism for adjusting head orientation movement toward a target.

## 2 | Materials and Methods

### 2.1 | Subjects and Ethical Approval

Sixteen adult male albino Wistar rats, each weighing between 300 and 350 g, raised in the University of Ferrara animal house, were used for this study. Five rats underwent bilateral long whiskers trimming and five rats bilateral short whiskers shaving. These 10 rats were recorded from the side. The remaining six rats, three bilaterally trimmed and three bilaterally shaved, were recorded from the front. The present research was based on two experiments each using a group of eight conditioned rats. In the first experiment, only the mystacial long whiskers on the pad have been bilaterally removed, leaving untouched the microvibrissae on the snout/upper lip, the lower jaw skin surface, and the eyebrow. In the second experiment, the microvibrissae and the embedded fur localized on the snout/upper lip have been bilaterally shaved. In the experiments, we used only male rats to avoid a bias in kinematic values between animals of different sex and size. During the experiments, execution blinding was not used because the type of whisker damage was objectively observable by the experimenters who provided the pellet on the shelf and video-recorded the rat behavior. Instead, the blinding of the experimenter at various stages in data analysis was carefully applied in the present study. The experimental plan was designed in compliance with Italian law regarding the care and use of experimental animals (DL26/14) and approved by both the University of Ferrara Ethics Board (OBA) and the Italian Ministry of Health. All procedures complied with the ethical standards regarding the treatment of animals in research issued by the European Council in its Directive dated 4 March 2014.

### 2.2 | Feeding and Food Restriction

Rats were housed in groups of three or four in polycarbonate cages (53 cm long, 37 cm wide, and 21 cm deep) with sawdust bedding, under a 12 h:12 h light/dark cycle with light starting at 07:30 h. All testing and training were performed at the same time of day during the light phase of the cycle. The animals received water ad libitum but were food-deprived before the start of training. The week before training began, each rat received 20 banana-flavored round food pellets (Rodent Tab 45 mg, AIN-76A, TestDiet, Richmond, VA, USA) 1 h prior to their daily fodder ration. Identical pellets would later serve as reaching targets in a single-pellet reaching box. Each animal maintained about 90% of their initial body weight throughout the experiment; to maintain body weight, the rats were given an additional amount of food in their cages after finishing their training or testing session.

### 2.3 | Reaching Box and Single-Pellet Training

The reaching box was made of clear Plexiglass (340 × 390 × 134 mm wide) and was similar to that described by Metz and Whishaw (2000) and Alaverdashvili et al. (2008). Briefly, the middle of the front wall featured a 10-mm-wide, vertical opening to allow the animal to reach for the pellet. This was placed on a shelf, 15 mm wide and 20 mm long, which was attached outside the front wall, at the level of the lower edge of the slot, 25 mm above the base. The upper surface of the shelf, aligned with the midline of the box, featured a round indentation (diameter, 7 mm; depth, 2.5 mm; distance from the front wall, 10 mm) for food pellet positioning. In this way, the pellet could be reached only by forepaw. During pretraining (about 1 week) each rat was placed in the box for 20-min daily sessions during which it was allowed to explore the reaching box and encounter the food pellets placed on the shelf to promote reaching through the slot and grasping. Pretraining ended when the rat began to reach for the food pellet using its forepaw. The subsequent training consisted of 15-min daily sessions during which the rat learned to grasp the pellet with their preferred paw. Paw preference was established when at least 60% of a minimum of 10 reach attempts were made using the left or right forepaw. During training, the rat was taught to advance from the posterior part of the box to the front wall, to poke its nose through the slot, and to identify the shelf, performing the prehension sequence after having recognized the presence of the pellet by sniffing. In order to facilitate learning of this movement sequence, a food pellet was dropped in the posterior part of the box in the initial training sessions. The prehension success was scored in the last week of training and during the following recording sessions. The percentage success rate of each rat was calculated as the ratio between the prehension movements in which the rat brought a food pellet to its mouth and the total number of trials multiplied by 100. For each rat, training ended when the percentage success rate exceeded 50% in three consecutive sessions and Control recordings began. As foreseen by the general experimental plan outline (Figure 1A), video recordings of control rats were made on two consecutive days before whisker manipulation. Trimmed and shaved rats were video-recorded on the same day as the intervention (Session 1) and the day after (Session 2), as 2 days were seen to be sufficient for assessing their recovery.

### 2.4 | Long Whiskers Trimming and Short Whiskers Shaving

Each day of training began with a 5–10 min handling session in which rats were conditioned to tolerate being held firmly while their vibrissae were touched by a set of blunt-tipped scissors. This conditioning enabled us to cut off the whiskers without anesthesia. When the rats were considered ready for the experimental procedure, they were recorded in two consecutive daily sessions as control. To distinguish macrovibrissae from microvibrissae, we have followed the criteria as defined in Brecht et al. (1997). Overall, the whiskers' length and their location around the snout were a key parameter in vibrissae discrimination. The transition between macrovibrissae and microvibrissae was not abrupt, but by using a surgical lens, a transition edge can be identified. Then, with the aid of a surgery lens, either the long mystacial whiskers were bilaterally trimmed to $<2$ mm in length or the short whiskers were shaved close to the skin.

### 2.5 | Video-Recording Rat Behavior

Throughout each experimental session, rats were video-recorded at 200 frames/s using a JVC GC-PX100 camera at a resolution of 640 × 360 pixels. In 10 animals, the recording video camera was positioned so as to obtain a right or left lateral view of the animal inside the box, according to the handedness of the animal. Moreover, to better evaluate how the animal approached the front wall, in six animals the video camera was positioned so as to obtain a frontal view of the animal inside the box. Recorded video clips were visualized offline, and frame-by-frame analysis was performed using Avidemux 2.6 software. Our previous observations in the oral grasping task (Parmiani et al. 2023) had revealed that trimmed and shaved rats adjust their orientation very rapidly from trial to trial so the present recordings were made immediately after trimming/shaving and on the day after.

### 2.6 | Analysis of Rat Behavior

Skilled reaching is a complex movement composed of three successive behavioral components: orientation, transport, and withdrawal, which could potentially be influenced by long and/or short whisker input. The aim of our research was to define the respective roles of the long and short whiskers in the orientation phase of skilled reaching. In our analysis of Orientation behavior, the trial start and end respectively coincided with the long whiskers' first contact with the front wall and the nose elevation (Figure 1B), to make space for the forepaw to cross the slot (Parmiani et al. 2019). After the long whiskers touched the front wall, the rat approached it and

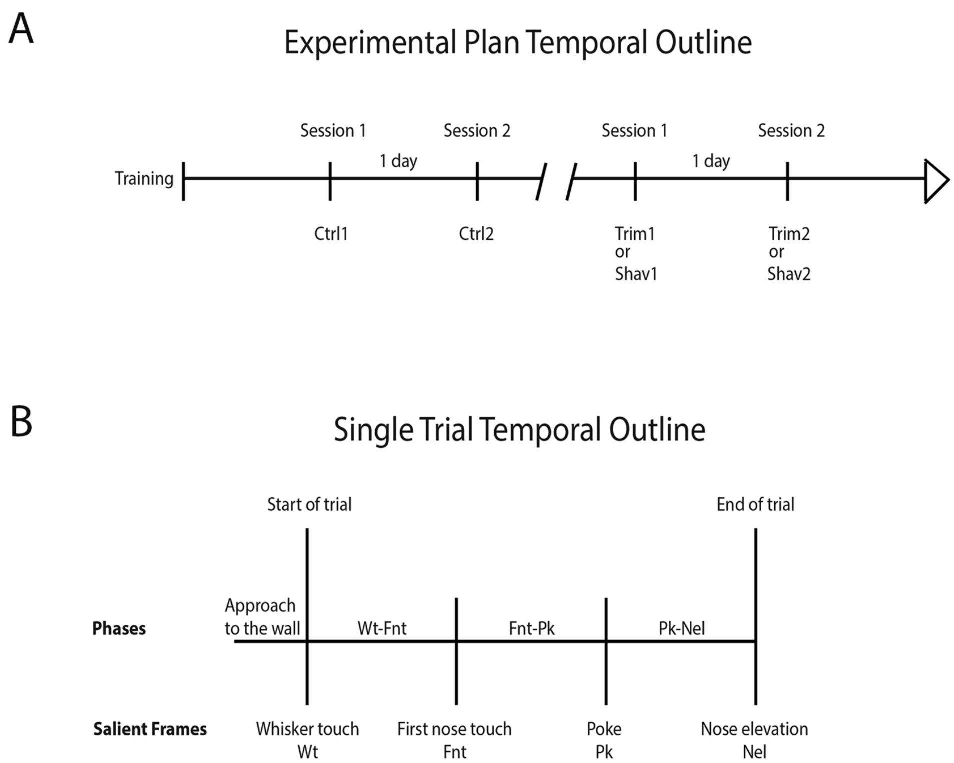


**FIGURE 1** | Diagram showing the time frame of the experimental plan (A) and a single trial (B). (A) Vertical bars indicate the temporal sequence of recording sessions and experimental procedures (trimming or shaving) in each animal. (B) Video analysis of salient frames and behavioral phases within a single trial. Fnt: first nose touch; Fnt–Pk: first nose touch–poke; Nel: nose elevation; Pk: poke; Pk–Nel: poke–nose elevation; Wt: whisker touch; Wt–Fnt: whisker touch–First nose touch.

located the slot by one or more nose touches to the wall, and then poked the nose into the slot. In some trials, the rat arrived so close to the slot that its nose directly crossed it at the level of the shelf, without head adjustment. As the nose poked through the slot, the long whiskers remained in contact with the inner surface of the front wall, whereas the short whiskers on the upper lip touched both the slot and the shelf; the rat sniffed to detect the presence of the pellet, and then, crossing the slot with its raised nose, lifted its preferential paw from the floor and carried out the reaching phase of the task. In that behavioral setup, the rat used olfaction to recognize the presence of the pellet on the shelf and did not touch it with its whiskers before reaching and grasping it (Whishaw and Tomie 1989; Parmiani et al. 2019).

Video frame analysis began with the detection of salient frames, i.e., those instants that allowed the definition of time intervals characterizing the rat behavior in control and injured animals. Figure 1B illustrates the conduction of a single trial, and presents the abbreviations for all the variables used throughout this paper. In sequence, the frame in which the long whiskers made contact with the front wall, defined as “whisker touch” (Wt; Figures 1B and 2A1), corresponded to the start of the trial. The next salient frame was that in which the nose contacted the front wall, defined as “first nose touch” (Fnt; Figures 1B and 2A2), which was followed by the frame in which the nose passed through the slot, defined as “poke” (Pk; Figures 1B and 2A3), and finally, the frame in which the nose rose from the shelf plane, defined as “nose elevation” (Nel; Figures 1B and 2A4). For each trial, we also determined the number of nose touches. For the transport phase, we identified the first frame, in which the paw lifted off the floor, i.e., “reach start” (Figure 2A5), and the last, when the paw crossed the slot, defined as “reach end” (Figure 2A6). Data related to reaching are not presented in the Results section, the focus being on the orientation interval between whisker touch and nose elevation, (Wt–Nel), which was a measure of the duration of orientation. Orientation was subdivided into three phases, viz., Wt–Fnt, Fnt–Pk, and Pk–Nel (Figures 1B and 2A1–6). Unlike Parmiani et al. (2018), who gave the rats 15–20 min to spontaneously explore the reaching box and locate the shelf, in the experiments presented here, the recordings were begun immediately after injury, without a habituation period inside the box.

## 2.7 | Data Analysis

A custom-made built-in script within MATLAB (MathWorks; Natick, MA) and R language (www.R-project.org/) was used for data analysis and to construct figures and statistical tests. Data were represented as medians and their interquartile ranges of $n$ determinations or MAD (median absolute deviation). For paired groups behavioral parameters, aligned rank transform (ART) for nonparametric repeated measures factorial ANOVA (Wobbrock et al. 2011) followed by the ART-C post hoc contrast tests (Elkin et al. 2021) was used to determine statistically significant differences between experimental group values. When performing post hoc multiple comparisons, the Holm $p$ value correction method for multiple hypotheses was applied. A significance level of $\alpha = 0.05$ was set for

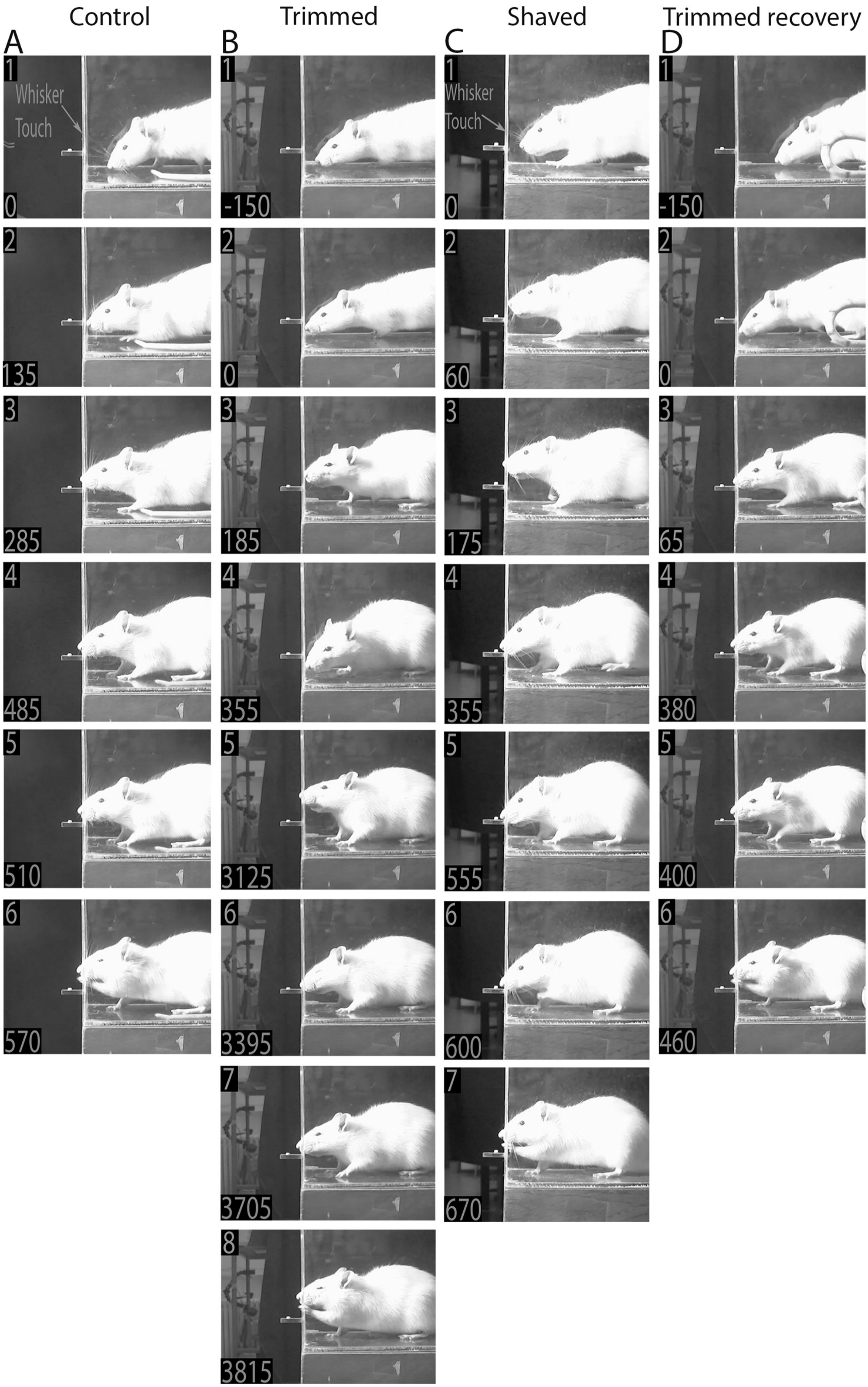


**FIGURE 2** | Legend on next page.

**FIGURE 2** | (A) Example of orientation in control skilled reaching trial: video recording from Rat 3. Each frame represents a salient step of the trial sequence: 1: whisker touch and trial start, 2: first nose touch, 3: poke, 4: nose elevation, 5: reach start, and 6: reach end. (B) Example of orientation loss in skilled reaching trial after bilateral trimming: video recording from Rat 3, Session 1, trial number 3. Each frame represents a salient step of the trial sequence. 1: approach to the front wall with head down posture, 150 ms before the first nose touch; 2–4: repeated nose touches; 5: poke; 6: nose lowering; 7: nose elevation and reach start; and 8: reach end. Note that in Frame 5, the poke takes place in a higher position than in control, and in Frame 6, the rat lowers the nose down toward the shelf. In Frame 7, the nose elevates synchronously with the reach start. (C) Example of orientation in skilled reaching trial after bilaterally shaving: video recording from Rat 2, Session 1, trial number 3. Each frame represents a salient step of the trial sequence: 1: whisker touch; 2: first nose touch; 3: poke; 4: shelf contact; 5: nose elevation; 6: reach start; and 7: reach end. Note that, when the animal pokes, its nose enters high and then drops down until it touches the shelf (Frames 3 and 4). (D) Example of orientation recovery in skilled reaching after bilaterally trimming: video recording from Rat 3, Session 2, trial number 3. Each frame represents a salient step of the trial sequence: 1: approach to the front wall with head down posture, 150 ms before first nose touch; 2: first nose touch; 3: poke; 4: nose elevation; 5: reach start; and 6: reach end. In each frame, the number at the bottom left is the timing (in milliseconds).

all tests. Pearson's method was used to compute correlation coefficients, whereas Levy's modification of Dunnett's test (Levy 1977) was used to compare correlation coefficients between the various experimental groups. DeepLabCut markerless motion tracking software was used to extract the rat's 2D nose position throughout each experimental session recording (Mathis et al. 2018).

## 3 | Results

### 3.1 | Orientation in Control Rats

The results presented below were derived from a total of 1000 recorded trials by 10 rats on two successive daily sessions (Ctrl1 vs. Ctrl2: day of first and second sessions, respectively), each one involving 50 trials per rat. Based on our previous experiments, two criteria defined a good level of training: (i) making 90% of reaching movement with the dominant forepaw and (ii) at least 50% of trials having a successful outcome. This is the reason why there were no significant differences among recording sessions in the same animal. The numerical values shown in the figures and in text are median ± MAD or frequencies in percentage. Tests used for the comparison were as follows: ART-C post hoc contrast tests after ART for nonparametric factorial ANOVA.

Frame-by-frame video analysis revealed four salient frames that defined the three successive phases of rat orientation behavior. The salient frames corresponded to consecutive events related to a particular use of an effector in tactile discrimination (long whiskers or macrovibrissae; nose/snout sensitive region; short whiskers or microvibrissae), invariably present in all trials in each rat (Figure 2A1–6). As the rat approached the front wall, the snout was in a downward posture with the whiskers in the protrusion phase of whisking (Figure 2A1). At this stage, the four consecutive salient frames were defined: (i) when the long whiskers came into contact with the front wall (whisker touch: Wt; Figure 2A1); (ii) when the rat, with the appropriate head movement, brought its nose into contact with the front wall (first nose touch: Fnt, Figure 2A2); (iii) when the rat inserted its nose through the slot (poke: Pk, Figure 2A3); and (iv) when the rat raised its nose (nose elevation: Nel, Figure 2A4) to allow its forepaw to pass through the slot to carry out the reaching movement (Figure 2A5–6). These four salient frames allowed orientation movement to be divided into three consecutive phases: (i) Wt–Fnt phase, giving a measure of the rat's ability to explore the front wall and slot position through prevailing long-whisker-mediated sense of touch (Figure 2A1–2); (ii) Fnt–Pk phase, giving a measure of the rat's ability to explore the front wall with its nose until this was poked through the slot (Figure 2A2–3); and finally (iii) Pk–Nel phase, giving a measure of the rat's ability to locate the shelf using tactile input from the snout/short whiskers and to verify the presence of the pellet by sniffing (Figure 2A3–4). During the Fnt–Pk phase, the rat searched for the slot by a number of nose touches varying in each trial between 0 and 2 (mean: 1.35 ± 0.13; trials with 0 nose touches: 9.6 ± 4.27%, $n = 1000$). No significant difference in phase duration was found between sessions (Ctrl1 vs. Ctrl2, trial duration: 370 ± 81.54 vs. 375 ± 74.13, Figure 3A1; Wt–Fnt: 110 ± 29.65 vs. 100 ± 29.65, Figure 3A2; Fnt–Pk: 140 ± 59.30 vs.140 ± 44.48 ms, Figure 3A3; Pk–Nel: 105 ± 66.72 vs.120 ± 66.72 ms, Figure 3A4; $p > 0.05$, $n = 1000$). These results, which also act as internal control, show that there was no statistical difference between sessions on Day 1 and Day 2 in controls, despite the intersubject variability. In fact, the control rats executed all phases in 100% of the trials and successfully grasped the pellet in 75.40 ± 2.72% of trials. All values were consistent with those obtained in control rats in previous experiments (Parmiani et al. 2018; Pellicer-Morata et al. 2021). These results confirmed that salient frames and derived intervals are behavioral invariants linked to the correct execution of the task.

### 3.2 | Orientation After Long-Whisker Trimming

In the session immediately after trimming (Trim1), rats lost the ability to direct their head toward the front wall, especially toward the slot. The approach to the wall was effected without slowing down, so the nose hit the wall as it made contact near the slot (Figure 2B1–2); the nose poked into the slot only after repeated attempts (Figure 2B2–5). In Trim1 rats, 7.6% of the trials (n. range: 0–11 in five rats) resulted in a lack of poke, because the rats were not able to detect the slot. These incomplete trials were observed in the context of the first trials in Session 1.

To highlight the short-term effects of whisker trimming, we compared data from Trim1 vs. Ctrl1 sessions. As noted above, trimmed rats' ability to detect the slot was impaired, with the trial beginning at the Fnt frame (Figure 2B2), rather than Wt. Nevertheless the trial duration decreased significantly (Trim1 vs. Ctrl1: 355 ± 140.85 vs. 375 ± 81.54 ms; $p = 0.005$, $n = 500$, Figures 2B1–8 and 3B1). Considering the two phases comprising

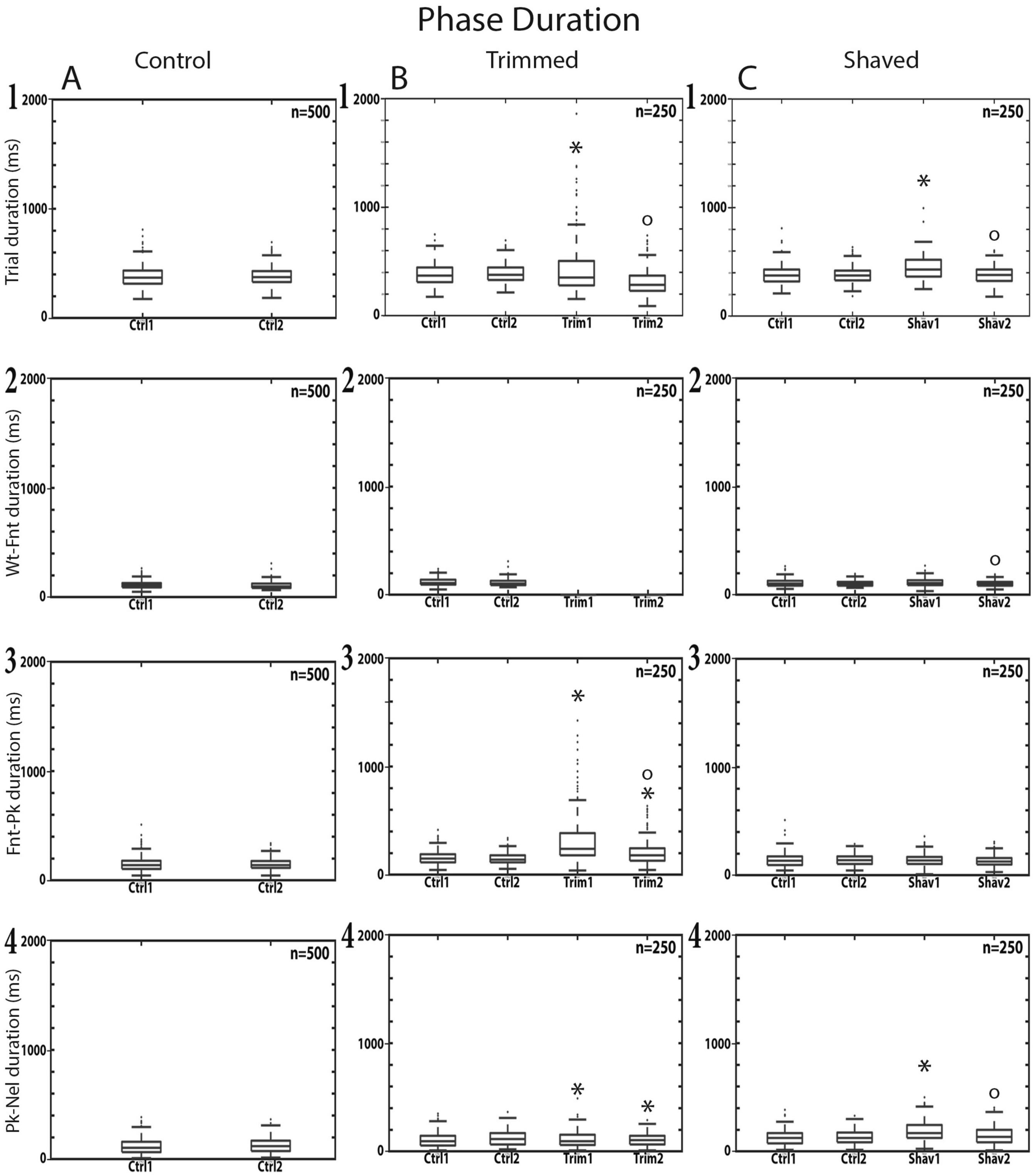


**FIGURE 3** | (A) Box plots of behavioral phase durations in 10 control rats; values are expressed as median ± IQR. 1: Trial duration, 2: Wt–Fnt duration, 3: Fnt–Pk duration, and 4: Pk–Nel duration. $n = 500$ trials in 10 rats for each box plot. No significant difference was found between Ctrl1 vs. Ctrl2. (B, C) Box plots of behavioral phase durations in control vs. trimmed (B, 1–4) and shaved rats (C, 1–4). Values are expressed as median ± IQR. (B1, C1) Trial duration, (B2–C2) Wt–Fnt duration, (B3–C3) Fnt–Pk duration, and (B4–C4) Pk–Nel duration. The asterisk (*) indicates a significant difference between experimental rats vs. corresponding controls; the small circle (°) indicates a significant difference between experimental rats in Session 2 vs. Session 1; $n = 250$ trials in five rats for each box plot.

orientation after trimming separately (Figure 2B2–6), we found a highly significant increase in duration of the Fnt–Pk phase (Trim1 vs. Ctrl1: 240 ± 118.61 vs. 135 ± 59.30 ms; $p < 0.0001$, $n = 500$, Figure 3B3) and a small but statistically significant decrease in the duration of the Pk–Nel phase (Trim1 vs. Ctrl1: 95 ± 66.72 vs. 125 ± 74.13 ms; $p < 0.0001$, $n = 500$, Figure 3B4).

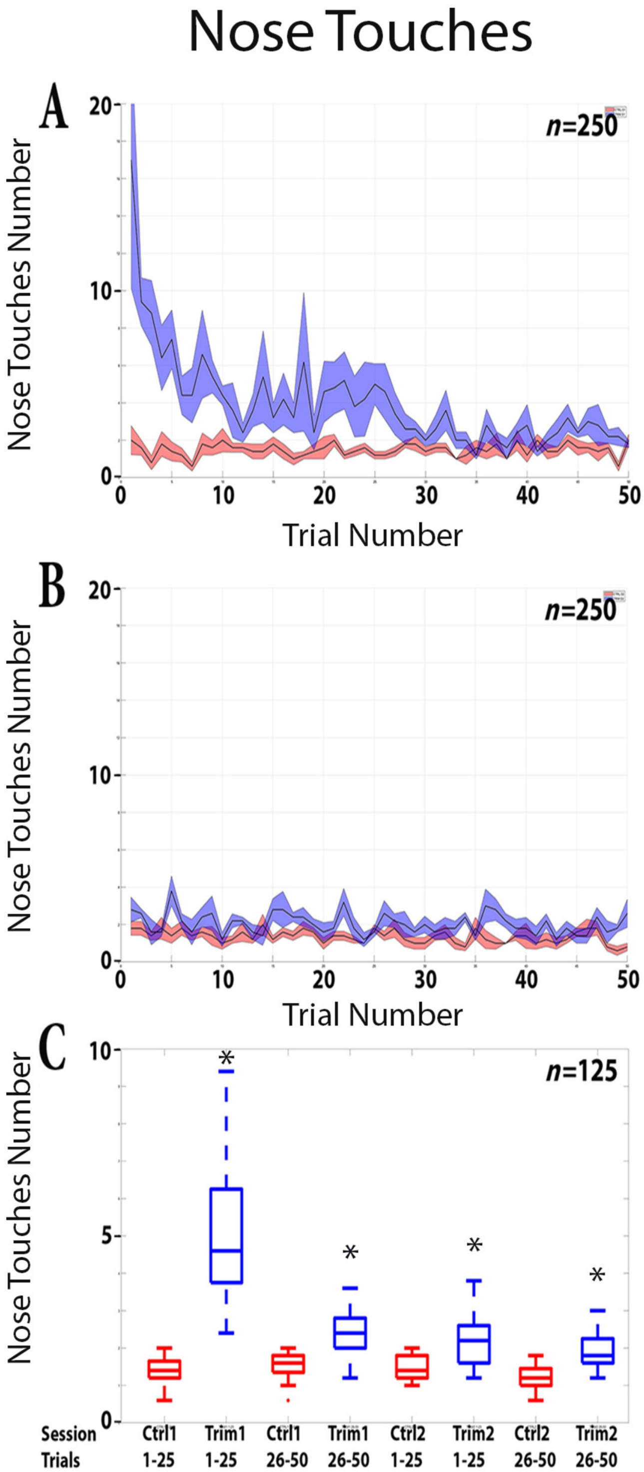


**FIGURE 4** | Legend on next page.

The recordings reveal that repeated nose touches are the main strategy rats use to compensate for alterations in their long-whisker-mediated sense, with several nose contacts with the wall being necessary to identify the slot position. Trim1 rats located the slot through repetitive nose touches (Figure 2B2–4), thereby lengthening the Fnt–Pk phase (Figure 3B3). Indeed, the mean number of nose touches in Trim1 trials was significantly higher in comparison to Ctrl1 trials (Trim1 vs. Ctrl1: $3.8 \pm 1.38$ vs.$1.54 \pm 0.32$; $p < 0.0001$, $n = 500$) and was found to be directly related to the duration of the Fnt–Pk phase ($R = 0.82$; $p < 0.0001$; Pearson's moment correlation coefficient). The time effects described above were observed in all animals but varied in size and duration depending on the individual animal.

**FIGURE 4** | Nose touch analysis. Mean number of nose touches, trial by trial (1–50) in five rats, control (blue) and trimmed (red), reported for Session 1 (A) and Session 2 (B). Note that the number of nose touches was highest in the first trials after trimming and progressively reduced, trial by trial, until trimmed values overlapped with control values at about the 25th trial. A significant difference was found for the first 10 trials in Session 1. (C) Box plots of the number of nose touches in Trim1, Trim2, Ctrl1, and Ctrl2, each one comprising 50 trials, divided into two groups of 25 successive trials, to compare groups homogeneous by trial number and recording time frame. Values are expressed as median ± MAD. $n = 125$ trials in five rats for each box plot. The asterisk (*) indicates a significant difference between experimental rats vs. corresponding controls.

The above results indicate that loss of orientation was selectively defined by alterations in three behavioral parameters: the presence of incomplete trials, longer duration of Fnt–Pk phase, and increased number of nose touches. Another observational outcome, confirmed by frame-by-frame analysis, was that the increase in the number of nose touches was greatest in the first trials after trimming and progressively reduced, trial by trial. The medium number of nose touches per trial (1–50) are reported for five rats, before and after trimming. In Session 1 (Figure 4A), statistical analysis of each trial showed that the difference in the number of nose touches between trimmed vs. control was significant from Trials 1 to 10 ($p < 0.001$), whereas in later trials, the difference was high but not statistically significant, except for Trials 18 and 22. Conversely, in Session 2, the number of nose touches by trimmed and control rats largely overlapped throughout the series ($p > 0.05$, Figure 4B). To explore this progressive reduction in nose touches, we divided Trim1, Trim2, Ctrl1, and Ctrl2 trials (50 trials each) into two groups of 25 consecutive trials each. This enabled a comparison of groups that were homogeneous by both trial number and recording time frame. The resulting box plots (Figure 4C) illustrate that after trimming, the first group showed a very significant increase in nose touches during the 1–25 trials in Session 1 as compared to control (Trim1 vs. Ctrl1 in trials 1–25: $5.10 \pm 2.01$ vs. $1.56 \pm 0.35$; $p < 0.005$, $n = 500$). A comparison of nose touches by the Trim1 group in Trials 1–25 vs. 26–50 revealed a large decrease in the second trial period (Trim1 1–25 vs. Trim1 26–60: $5.10 \pm 2.01$ vs. $2.50 \pm 0.76$; $p < 0.005$, $n = 500$).

To more convincingly demonstrate the profound loss of spatial perception after long whisker trimming, we plotted the nose touches on a Cartesian plane representing the front wall of the reaching box, with the pellet position as the origin. By means of a markerless technique, in the three rats recorded from the front we were able to pinpoint the position of the nose touches with respect to the pellet in each trial. See, as examples, a control trial, which featured two nose touches, as compared to one of the first trials after trimming, with five nose touches (Figure 5A–C). The plots clearly reveal that after trimming the individual nose touches appeared randomly scattered on the front wall around the slot. This is even more evident from

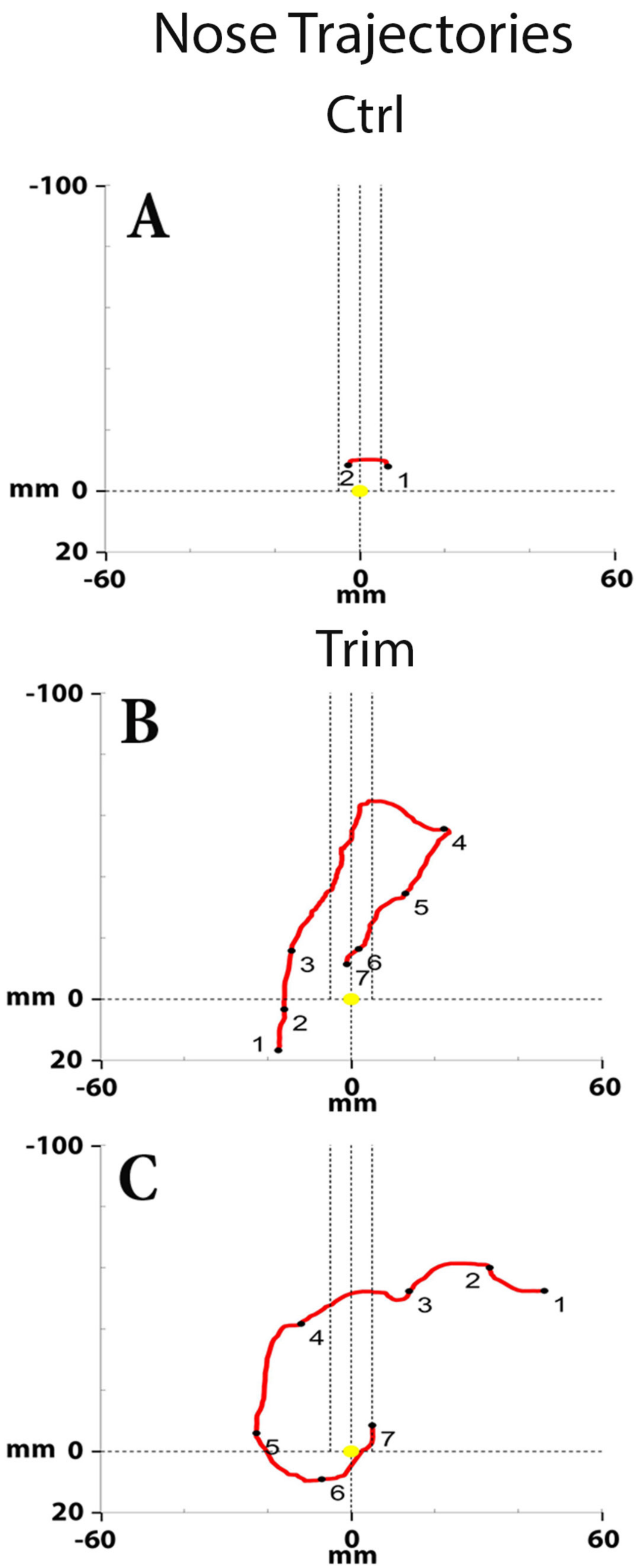


**FIGURE 5** | Example of nose trajectory in control and trimmed rats. Single trajectory from first to last nose touch before poking in the frontal recording. Each dot corresponds to a nose contact with the front wall and the numbers indicate the sequence of touches. Video recording from Rat 4, Session 1, trial number 4 before and after trimming. Note that after trimming the rat requires several nose touches to locate the slot.

the first nose touch scatter plots, which illustrate the first nose contact with the front wall in 75 trials from Ctrl1 (Figure 6A) and 75 trials from Trim1 1–25 (Figure 6B). The scatter plots were used to calculate both the area of the ellipse that included 95% of the data values and the barycentric position: The former measure indicated how close the data points were to one another, i.e., the rat's orientation precision, and the latter the position of the head with respect to the pellet. They show that the first nose touches by control rats were distributed very close to each other around the pellet, describing an ellipse characterized by an area of 284.82 $mm^2$ (minor axis = 8.48 mm, major axis = 10.69 mm) and a barycenter of $X = 0.22$ mm and $Y = 7.99$ mm. We assumed that this condition was of high precision and the best head position with respect to the pellet (Figures 6A,E). In Trim1 1–25, nose touches were contained within an ellipse of area 4244.17 $mm^2$, 15 times greater than that of control rats. They revealed a more pronounced loss of precision along the major axis (48.95 mm) with respect to the minor axis (27.60 mm) as shown in Figure 6B. As for the barycenter, the $X$ coordinates did not change statistically, whereas the $Y$ coordinates statistically decreased, suggesting that the head position on the vertical axis was lower than in controls (Trim1 1–25 vs. Ctrl1, $X$ axis: −3.75 vs. 0.22 mm, $p > 0.05$; $Y$ axis: 1.12 vs. 7.99, $p < 0.005$, $n = 150$; Figure 6B,E). Nose touch distribution symmetry was therefore maintained on both axes, despite the greater dispersion on the $X$, and the barycenter lower down on the $Y$.

### 3.3 | Orientation After Short-Whisker Shaving

Immediately after shaving (Shav1), rats maintained their ability to direct their head toward the front wall, especially toward the slot. Unlike Trim trials, 100% of Shav1 trials featured a successful nose poke, and orientation was not grossly impaired. As for the sequence of phases that comprised the trials, it was the same as described for Ctrl1: four salient frames delimiting three distinct phases. Detailed video analysis showed that, unlike in Ctrl1 and Trim1 trials, the Pk–Nel phase in Shav1 trials involved the lip/snout being repeatedly pressed over the shelf, maintaining prolonged contact with it (Figure 2C2–5). This accounts for the statistically significant increase in trial duration seen in Shav1 (Shav1 vs. Ctrl1: 430 ± 103.78 ms vs. 375 ± 81.54, $p < 0.0001$, Figure 3C1), $n = 500$). Indeed, when considering the three orientation phases separately (Figure 2C1–5), the duration of the Pk–Nel phase was found to be highly significantly increased with respect to control (Shav1 vs. Ctrl1: 170 ± 81.54 vs. 125 ± 74.13 ms; $p < 0.0001$, $n = 500$, Figure 3C4), whereas the respective durations of the Wt–Fnt and Fnt–Pk phases were unaffected (Wt–Fnt: Shav1 vs. Ctrl1: 110 ± 37.06 vs. 105 ± 29.65 ms; $p > 0.05$, Figure 3C2; Fnt–Pk: Shav1 vs. Ctrl1: 137.5 ± 48.18 vs.135 ± 59.30 ms; $p > 0.05$ $n = 500$, Figure 3C3). Note, however, that there was no difference between mean number of nose touches in shaved vs. control rats (Shav1 vs. Ctrl1: 1.60 ± 0.38 vs. 1.24 ± 0.29, $p > 0.05$, $n = 500$). Such time effects were observed in each animal, and the above results indicate that the impact of short-whisker removal selectively regarded two behavioral parameters: the trial as a whole and Pk–Nel duration.

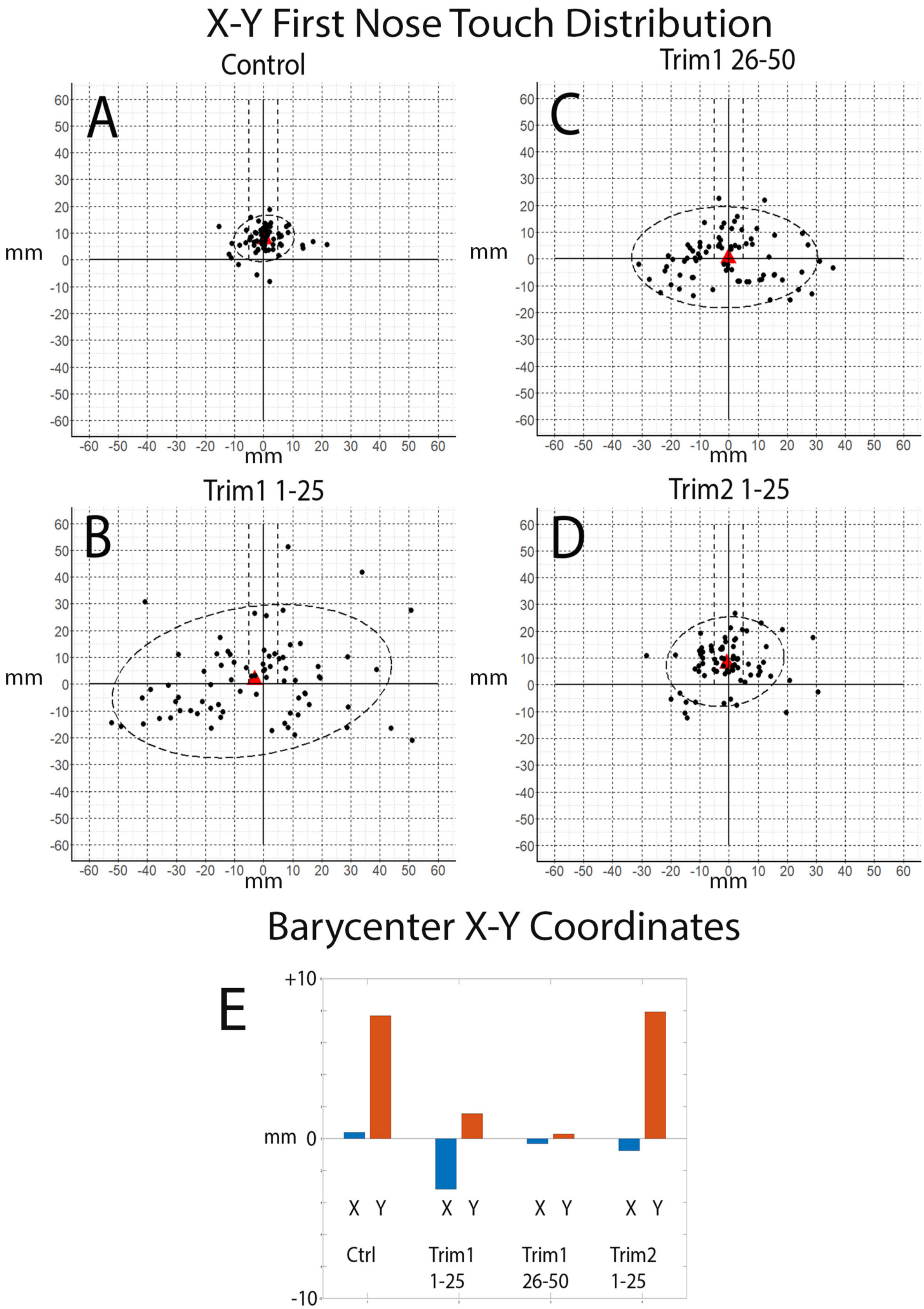


**FIGURE 6** | Legend on next page.

**FIGURE 6** | First nose touch position distribution in control rats and after trimming. In this distribution, each point corresponds to the first nose contact with the front wall in each single trial. Data derived from 25 trials of three rats video-recorded from the front and was gathered under control conditions (A) and after trimming: (B–D). *X* axis: corresponds to the medial–lateral dimension of the front wall, positioned at the shelf level; *Y* axis: corresponds to the vertical dimension of the front wall, positioned on the midline; 00: corresponds to the pellet on the shelf. Broken lines correspond to the vertical border of the slot. In each plot, the ellipse encloses 95% of nose touches and the barycenter is marked. Note that the ellipse is smaller in controls, enlarged maximally immediately after trimming, and shrinks progressively in C and D as the session continues. (E) Histogram of *XY* coordinates of ellipse barycenters. Coordinates on the *Y* axis are reported in millimeters as positive or negative values for Ctrl, Trim1 1–25, Trim1 26–50, and Trim2 1–25. Note that only *Y* coordinates show significant differences in Trim1 1–25 and Trim1 26–50.

### 3.4 | Recovery of Orientation Capacity

Observation of trimmed rats' behavior revealed that they gradually and partially regained orientation movement—recovering their ability to direct their head toward the front wall and, importantly, the slot—during the first day's session (Figure 2D1–6). To quantify the recovery 1 day after trimming, data analysis was performed, comparing Trim2 vs. Trim1 and Trim2 vs. Ctrl2. This showed that the Fnt–Pk duration was statistically significantly lower, by ~25%, on the second vs. the first day (Trim2 vs. Trim1: 180 ± 81.54 vs. 240 ± 118.61 ms; $p < 0.0001$, $n = 500$; Figure 3B3) but did not reach the baseline value represented by the control trials (Trim2 vs. Ctrl2: 180 ± 81.54 vs. 140 ± 51.89 ms; $p = 0.02$, $n = 500$; Figure 3B3).

The reduction in nose touches number between the first and second subsessions on Day 1 suggests a rapid recovery of the rat's ability to orient toward the slot (Trim1 1–25 vs. Trim1 26–50: 5.10 ± 2.01 vs. 2.50 ± 0.76, $p < 0.0001$, Figure 4C). On the second day, the rats' ability to locate the slot improved by ~54% with respect to the first day (Trim2 vs. Trim1: 2.04 ± 0.56 vs. 3.8 ± 1.38; $p < 0.001$, $n = 500$). Nevertheless in Session 2, trimmed rats still required a significantly greater number of nose touches to locate the slot than the corresponding control (Trim2 vs. Ctrl2, Trials 1–25: 2.14 ± 0.65 vs. 1.31 ± 0.27; $p < 0.0001$; Trials 26–50: 1.95 ± 0.48 vs. 1.23 ± 0.35; $p < 0.0001$; $n = 500$), with no significant difference between the first and second halves of the session (2.14 ± 0.65 for Trials 1–25 vs.1.95 ± 0.48 for Trials 26–50; $p > 0.83$, $n = 500$, Figure 4C).

Regarding the distribution of nose touches, in trimmed rats, these were confined to an ellipse of area 1909.19 $mm^2$ (minor axis = 18.86 mm, major axis = 32.22 mm) in Trials 26–50 on Session 1. This is about half the size of the ellipse area generated in Trials 1–25 from the same session (4244.17 $mm^2$) but 6.7 times as wide as that seen in control rats. The axes' lengths were also reduced, along with the area, but the distribution symmetry was maintained (Figure 6C). On the second day, over Trials 1–25, the ellipse area was 1066.43 $mm^2$ (minor axis = 16.47 mm, major axis = 20.61 mm), remaining 3.7 times larger than control (Figure 6D vs. A).

Regarding the barycenter coordinates, there was no statistically significant difference on the *X* axis between Trials 1–25 and 26–50 on Session 1 in trimmed rats, whereas the *Y* coordinate remained statistically significantly different to controls, suggesting a persistently lower head position on vertical axis (Trim1 26–50 vs. Ctrl, *X* axis: −1.25 vs. 0.22, $p > 0.05$; *Y* axis: 0.58 vs. 7.99, $p < 0.005$, $n = 150$; Figure 6C vs. A and E). Nose touch distribution symmetry was maintained on both axes but with a greater dispersion on the *X* axis. In Session 2, the *X* and *Y* coordinates in the first 25 trials were comparable with those for controls, demonstrating that the baseline barycenter had been regained (Figure 6D vs. A and E).

Shaved rats gradually regained the ability to detect the shelf over the course of Day 1. Their recovery was evaluated by means of the variable altered by short whisker removal, viz., the Pk–Nel duration. On Day 2, the Pk–Nel phase duration was reduced by ~21% of that recorded on Day 1, a significant difference (Shav2 vs. Shav1: 135 ± 88.96 vs. 170 ± 81.54 ms; $p < 0.0001$, $n = 500$, Figure 3C4). A comparison of the Pk–Nel duration recorded for shaved rats in Session 2 with that displayed in the second day of control trials revealed that it returned to baseline (Shav2 vs. Ctrl2: 135 ± 88.96 vs. 125 ± 66.71 ms; $p = 0.45$, $n = 500$; Figure 3C4).

### 3.5 | Orientation: Long-Whisker Trimming vs. Short-Whisker Shaving

To more convincingly determine the selective involvement of long and short whiskers in orientation phases, the data and their statistical significance were compared between trimmed vs. shaved rats. When considering the Trim1 and the Shav1, the duration of the Fnt–Pk phase was found to be highly significantly increased in Trim1 with respect to Shav1 rats (Trim1 vs. Shav1: 240 ± 118.61 vs. 137.5 ± 48.18 ms; $p < 0.0001$, $n = 500$, Figure 3B3 vs. C3). Similarly, the durations of Fnt–Pk was found to be significantly increased in Trim2 with respect to Shav2 rats (Trim2 vs. Shav2: 180 ± 81.54 vs. 127.5 ± 48.18 ms; $p < 0.0001$, $n = 500$, Figure 3B3 vs. C3). For what concern the Pk–Nel duration, this phase was found to be significantly lower in Trim1 with respect to Shav1 rats (Trim1 vs. Shav1: 95 ± 66.72 vs. 170 ± 81.54 ms; $p < 0.0001$, $n = 500$, Figure 3B4 vs. C4). Similarly, the durations of Pk–Nel was found to be significantly lower in Trim2 with respect to Shav2 rats (Trim2 vs. Shav2: 105 ± 59.30 vs. 135 ± 88.96 ms; $p < 0.001$, $n = 500$, Figure 3B4 vs. C4). These comparisons further support the conclusion that long and short whiskers are prevailingly engaged in different phases of the task sequence.

## 4 | Discussion

A common paradigm used to explore how sensory tactile inputs influence a rat's object location and recognition abilities in a controlled context is the skilled reaching task. This makes it possible to study the respective roles of the long and short whiskers in tactile discrimination. Skilled reaching is composed of three successive behavioral responses: orientation, transport, and withdrawal (Alaverdashvili and Whishaw 2013). In the present study, we focused our attention on the orientation response, which is more demanding than transport and withdrawal in terms of fine tactile discrimination. The notion that both long and short whiskers are engaged in tactile discrimination for effective orientation in any environmental context (Welker 1964; Brecht et al. 1997; Hartmann 2001; Prescott et al. 2011; Parmiani et al. 2023) prompted us to perform two complementary experiments to study the short-term effects of trimming the long whiskers and shaving the short whiskers, respectively, on orientation behavior as compared to control rats. We analyzed each trial from the moment the long whiskers first came into contact with the front wall of the reaching box to the point at which the nose, after having poked through the slot, raised to enable the reaching phase of the task.

By means of frame-by-frame analysis in control rats, we found that orientation could be subdivided, by mean of salient frames, into three behavioral phases: Wt–Fnt, Fnt–Pk, and Pk–Nel, corresponding respectively to the rat's ability to explore the front wall, to find the slot and nose poke through it and locate the shelf. As a whole, the analysis indicates that these phases were consecutive and invariable in duration, guided by sensory signals from the long whiskers, nose/snout, and short whiskers, supporting the idea that these three phases corresponded to three different computational steps (LeCun et al. 2015). Control rats needed very few nose touches (an average of 1.33 per trial) to gather the information necessary to identify the wall/slot position and make the decision to poke. The fact that the number of touches per trial varied little both within and between animals demonstrates that they relied on the same total quantity of evidence in each trial. Our data seem to suggest that the Wt–Fnt and Pk–Nel phases are mainly guided by the long and short whiskers, respectively, whereas the Fnt–Pk phase is guided by both the long whiskers and sensory signals from the nose/snout. Comparing behavioral data and parameter values between control and injured rats suggests that, in orientation sensory regulation, long whisker signals appear to guide the head/nose toward the correct position for nose insertion into the slot; then, nose/snout signals prompt correction of head movement to align it properly with the pellet, and short whisker signals enable identification of the shelf and its position (Prescott et al. 2011; Vale et al. 2017; Parmiani et al. 2018).

The whiskers' sensory and motor systems are linked at many anatomical levels by a set of closed sensorimotor feedback loops (Kleinfeld et al. 1999; Nguyen and Kleinfeld 2005; Bellavance et al. 2017) representing the main pathway where tactile information can drive motoneurons before projecting to higher levels of sensory and motor systems. These loops convey sensory information originating from the whiskers to facial and neck motor neurons, ensuring a finer control of whisking and head movement, coordinated with rhythmic motor activity of the face (Kurnikova et al. 2017). The presence of a high density of spindle receptors in the neck muscles makes it evident that such loops play an important role in the precise adjustment of head position (Pfister and Zenker 1984; Alstermark et al. 1992). Nguyen and Kleinfeld (2005) suggest that lower feedback may serve as a short latency regulation to optimize the whisking/head movements mechanics.

Thus, the question was raised as to whether orientation behaviors depend on cortical processes or not. Neuronal activity in the S1 cortex reveals an early sensory response (< 50 ms) to a tactile stimulus and a second, late one (50–400 ms) (Ferezou et al. 2009; Ferezou and Deneux 2017). Because the duration of the phases that make up the orientation movement is almost always greater than 100 ms, our data suggested that what we have observed are phenomena involving cortical activity. In line with previous anatomical and physiological data, our behavioral results suggested different roles for the two separate whisker subsystems, which independently served different specialized functions and together enabled animals to search for and recognize surrounding objects. The results obtained are related to changes in the whiskers' somatosensory and motor systems. Separate pathways of vibrissa information processing are designated as the lemniscal, extralemniscal, and paralemniscal pathways. The lemniscal and extralemniscal pathways relay vibrissa information through the ventral posteromedial nucleus of the thalamus defined as first-order relay nucleus, whereas the paralemniscal pathway projects through the posterior group (PO) of the thalamus regarded as higher order relay nucleus (Pouchelon et al. 2012; Deschenes and Urbain 2016; Casas-Torremocha et al. 2022). These two nuclei show differences in the properties of their neurons with the bilateral fields of PO neurons (Castejon et al. 2021). The PO is massively linked with the face and mouth-related region of the motor and somatosensory cortex (Casas-Torremocha et al. 2022). In the rat's somatosensory cortex, whiskers are represented in three individual barrel subfields that correspond to the three zones of the snout where the vibrissae are distributed (i) in posteromedial barrel subfield, for the mystacial whiskers; (ii) in anterolateral barrel subfield, for the short whiskers/hairs on the snout, buccal pad, and upper lip; and (iii) in lower jaw barrel subfield, for the whiskers/hairs on the lower jaw (Woolsey and Van der Loos 1970; Welker and Woolsey 1974; Thé et al. 2013; Pellicer-Morata et al. 2021). Then, it is possible that trimming and shaving could generate a topographical different change in thalamic barreloid activity that induces a reorganization of the corresponding posteromedial or anteromedial barrel subfields.

The suppression of a particular input is expected to induce effects proportional to the physiological role of that afferent in the control of the specific behavior in a multisensory context (Stein et al. 2014; Bean et al. 2023). Under our experimental conditions, behavioral changes when long and short whiskers were trimmed or shaved suggest that these different types of whiskers are involved in distinct phases of orientation behavior.

The long whisker trimming always led to a striking loss of orientation skills, coupled with a significant increase in the number of nose touches. Indeed, when their long whiskers were removed, the rats collected information about the wall and slot positions through repeated nose touches, i.e., the way they explored their peripersonal space changed, shifting from whisker- to a nose-mediated sense of touch (see also Parmiani et al. 2018), which resulted in a conspicuous slowdown in trial execution. A major finding of this study regards the 2D distribution of the first nose touches in trimmed vs. control rats. Although its long whiskers remained intact, the rats executed a coherent orientation movement characterized by first nose touches enclosed in an elliptical area (284.82 $mm^2$; major axis: 21.38 mm, minor axis:

16.96 mm) with a barycenter aligned with the target/pellet (coordinates, $X$: +0.22 mm and $Y$: +7.99 mm). This means that the rat approached the slot/shelf with the head already in the correct position to target the pellet, i.e., in line with the midline of the slot along the horizontal axis and slightly above along the vertical axis. The elliptical distribution plot clearly shows that, after the first nose contact with the front wall, the rat adjusts its head position in a range described by half the length of the ellipse axes. The correction of initial errors in the approach was within ~10 and ~8 mm on the major and minor axes, respectively. As a whole, these figures define the rat's "best practice" in terms of orientation performance. Long-whisker trimming, on the other hand, caused an approach error of 3.25–4.6 times higher than control values, with a corresponding loss of nose positioning precision. Nose touch distribution symmetry was, however, maintained on both axes, albeit with greater dispersion on the $X$ axes, with the barycenter lower down on the $Y$. These results suggest that in trained rats, the success of the orientation movement, whose end point is the pellet position, does not depend wholly on information received from the long whiskers, which is otherwise a fundamental guide for the rat in the executive phase of orientation. In other words, the rat knows where to go, but without access to vibrissal information, it is unable to rapidly correct its movement trajectory, and a greater number of corrective movements, each one corresponding to nose touch, is needed to successfully reach the target. This deficit can be interpreted as posterior cordonal ataxia, as defined in primates (Akbar and Ashizawa 2015). A greater dispersion of first nose touches on the $X$ axis could be ascribed to an anatomical reason: Given the distribution of long whiskers on the rat's muzzle, the tactile information they capture originates prevalently from the horizontal plane. The resultant shift of the barycenter down the $Y$ axis, on the other hand, could be due to the lack of tactile input physiologically reaching the neck motor neurons from the long whiskers.

Another key finding of this study is that after trimming, orientation skills were reacquired within approximately the first 6 min of Session 1, suggesting that the rat rapidly deployed a mechanism for spatial relearning (Luft and Buitrago 2005). The data reveal that the number of nose touches decreased trial by trial over the first session, coinciding with a reduction in trial duration, whereas it was not until the second session that the barycenter coordinates normalized. Regarding the recovery of parameters noted in Session 1, it might be argued that this could be due to the "warm-up" effect, i.e., an improvement in performance after practice (Bishop 2003; da Cruz et al. 2017), but there was no similar change in the number of nose contacts in the second session, casting significant doubt on this interpretation. However, the rapid change of the sensor from whiskers to nose tip that originates the salient input suggested a certain degree of adaptation that appeared very quickly and persisted until we recorded the animal behavior on Day 2 (Campagner et al. 2023).

The reorganization of the whiskers/neck movement representation in the motor cortex has been studied following facial nerve severing and repair (Huntley 1997; Franchi 2000a, 2000b) or motor vibrissae mapping at different levels of anesthesia (Tandon et al. 2008). As a whole, these experiments revealed a substantial overlapping representation of the neck and whiskers muscles in a large medial region of the motor cortex. This overlap suggests the M1 vibrissae as a cortical area involved in whiskers/neck coordination in orientation behavior. Then, the short-term reorganization of motor cortical circuits (Sanes et al. 1988; Donoghue et al. 1990; Franchi 2001) could be the cortical mechanism involved in the change of orientation behavior. Further evidence of short-term plasticity has been provided by previous research showing that rats bear head direction (HD) cells—in the anterodorsal thalamus and post-subiculum (Angelaki and Laurens 2020) that respond to visual cues; when their input was eliminated, the rats apparently relied on a rapid learning mechanism that enabled them to develop associations with landmark cues. In fact, 8 min of exposure to a novel visual cue was enough time to control the preferred firing direction of HD cells (Goodridge et al. 1998). Moreover, rapid spatial learning has been demonstrated in mice trained in a circular arena to escape aversive threats; to adapt to the changing environment, their orientation behavior was rapidly corrected in a single trial. This is indicative of the whisker system having a rapid memory capacity for different forms of behaviors (Vale et al. 2017). A similar mechanism could be involved in the fast orientation recovery seen in our experiment, with the information needed to complete the task being derived from the nose/muzzle in the absence of the long whiskers.

As regards shaving of the short whiskers, this did not induce a specific type of disorientation—as trimming the long whiskers did—but rather a delay in their identification/location on the shelf. This slowed the execution of the trial as a whole but to a lesser extent. It has already been shown that the identification of the shelf has a decisive trigger function for the next phase of reaching/grasping (Parmiani et al. 2018). The behavior we recorded suggests that shelf identification is performed via short whisker input in control conditions, but shaving the short whiskers caused the animal to rely on the tactile sensitivity of the muzzle instead of the microvibrissae to recognize the shelf by repeatedly nudging it. That being said, we cannot exclude a proprioceptive component from the neck muscle spindles and/or the vestibular system (Cullen 2012). Moreover, present comparisons make it clear the long whiskers' ability to orient head/nose movement toward the slot during the early stages of orientation. On the second day after trimming, rats partially regained orientation movement but only the barycenter reached the baseline values. Conversely, in shaved rats, orientation was not grossly impaired, but the short whiskers removal affected the ability to detect the shelf over the last phase of orientation, which on the second day returned to baseline. In conclusion, in line with previous anatomical and physiological data, our behavioral results also suggested different roles for the two separate whisker subsystems, which independently served different specialized functions and together enabled animals to search for and recognize surrounding objects. However, it cannot be excluded that the instantaneous reflex mechanisms may be supported by a subcortical system. Based on the concept that long whiskers code distance in fixed, head-centered coordinates, our future research will involve unilateral whisker trimming/shaving in order to study short-term asymmetry in explorative behavior (Meyer and Meyer 1992). Moreover, using the same experimental paradigm, we will undertake a kinematic investigation to better define any impairment in orientation movement.

## Author Contributions

**Pierantonio Parmiani:** conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, validation, visualization. **Cristina Lucchetti:** conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, writing – original draft. **Riccardo Viaro:** data curation, visualization. **Luciano Fadiga:** conceptualization, funding acquisition, writing – original draft. **Gianfranco Franchi:** conceptualization, investigation, methodology, project administration, supervision, validation, writing – original draft.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Raw data are available upon request to the corresponding authors.

## Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ejn.70169.

## References

Ahissar, E., and P. M. Knutsen. 2008. "Object Localization With Whiskers." *Biological Cybernetics* 98: 449–458.

Ahl, A. S. 1986. "The Role of Vibrissae in Behavior: A Status Review." *Veterinary Research Communications* 10: 245–268.

Akbar, U., and T. Ashizawa. 2015. "Ataxia." *Neurologic Clinics* 33: 225–248.

Alaverdashvili, M., H. Leblond, S. Rossignol, and I. Q. Whishaw. 2008. "Cineradiographic (Video X-Ray) Analysis of Skilled Reaching in a Single Pellet Reaching Task Provides Insight Into Relative Contribution of Body, Head, Oral, and Forelimb Movement in Rats." *Behavioural Brain Research* 192: 232–247.

Alaverdashvili, M., and I. Q. Whishaw. 2013. "A Behavioral Method for Identifying Recovery and Compensation: Hand Use in a Preclinical Stroke Model Using the Single Pellet Reaching Task." *Neuroscience & Biobehavioral Reviews* 37: 950–967.

Alstermark, B., M. J. Pinter, S. Sasaki, and B. Tantisira. 1992. "Trigeminal Excitation of Dorsal Neck Motoneurones in the Cat." *Experimental Brain Research* 92: 183–193.

Angelaki, D. E., and J. Laurens. 2020. "The Head Direction Cell Network: Attractor Dynamics, Integration Within the Navigation System, and Three-Dimensional Properties." *Current Opinion in Neurobiology* Neurobiology of Behavior 60: 136–144.

Arkley, K., R. A. Grant, B. Mitchinson, and T. J. Prescott. 2014. "Strategy Change in Vibrissal Active Sensing During Rat Locomotion." *Current Biology* 24: 1507–1512.

Bean, N. L., B. E. Stein, and B. A. Rowland. 2023. "Cross-Modal Exposure Restores Multisensory Enhancement After Hemianopia." *Cerebral Cortex* 33: 11036–11046.

Bellavance, M.-A., J. Takatoh, J. Lu, et al. 2017. "Parallel Inhibitory and Excitatory Trigemino-Facial Feedback Circuitry for Reflexive Vibrissa Movement." *Neuron* 95: 673–682.e4.

Bishop, D. 2003. "Warm Up I." *Sports Medicine* 33: 439–454.

Brecht, M., B. Preilowski, and M. M. Merzenich. 1997. "Functional Architecture of the Mystacial Vibrissae." *Behavioural Brain Research* 84: 81–97.

Calvert, G. A., and T. Thesen. 2004. "Multisensory Integration: Methodological Approaches and Emerging Principles in the Human Brain." *Journal of Physiology-Paris Representation of 3-D Space Using Different Senses in Different Species* 98: 191–205.

Campagner, D., R. Vale, Y. L. Tan, et al. 2023. "A Cortico-Collicular Circuit for Orienting to Shelter During Escape." *Nature* 613: 111–119.

Casas-Torremocha, D., M. Rubio-Teves, A. Hoerder-Suabedissen, et al. 2022. "A Combinatorial Input Landscape in the "Higher-Order Relay" Posterior Thalamic Nucleus." *Journal of Neuroscience* 42: 7757–7781.

Castejon, C., J. Martin-Cortecero, and A. Nuñez. 2021. "Higher-Order Thalamic Encoding of Somatosensory Patterns and Bilateral Events." *Frontiers in Neural Circuits* 15: 752804.

Cullen, K. E. 2012. "The Vestibular System: Multimodal Integration and Encoding of Self-Motion for Motor Control." *Trends in Neurosciences* 35: 185–196.

da Cruz, V. H., C. S. Peserico, and F. A. Machado. 2017. "Effect of Prior Warm-Up Duration on the Time Limit at Peak Speed in Untrained Men." *Journal of Sports Medicine and Physical Fitness* 57: 1276–1281.

Deschenes, M., and N. Urbain. 2016. "Vibrissal Afferents From Trigeminus to Cortices." In *Scholarpedia of Touch*, edited by T. Prescott, E. Ahissar, and E. Izhikevich, 657–672. Atlantis Press.

Diamond, M. E., and A. Toso. 2023. "Tactile Cognition in Rodents." *Neuroscience & Biobehavioral Reviews* 149: 105161.

Donoghue, J. P., S. Suner, and J. N. Sanes. 1990. "Dynamic Organization of Primary Motor Cortex Output to Target Muscles in Adult Rats II. Rapid Reorganization Following Motor Nerve Lesions." *Experimental Brain Research* 79: 492–503.

Elkin, L. A., M. Kay, J. J. Higgins, and J. O. Wobbrock. 2021. "An Aligned Rank Transform Procedure for Multifactor Contrast Tests." In *The 34th Annual ACM Symposium on User Interface Software and Technology, UIST'21*, 754–768. Association for Computing Machinery.

Ferezou, I., and T. Deneux. 2017. "Review: How Do Spontaneous and Sensory-Evoked Activities Interact?" *Neurophotonics* 4: 031221.

Ferezou, I., F. Matyas, and C. C. H. Petersen. 2009. "Imaging the Brain in Action: Real-Time Voltage-Sensitive Dye Imaging of Sensorimotor Cortex of Awake Behaving Mice." In *Vivo Optical Imaging of Brain Function*, edited by R. D. Frostig, 2nd ed. edn, Frontiers in Neuroscience. CRC Press/Taylor & Francis.

Franchi, G. 2000a. "Reorganization of Vibrissal Motor Representation Following Severing and Repair of the Facial Nerve in Adult Rats." *Experimental Brain Research* 131: 33–43.

Franchi, G. 2000b. "Changes in Motor Representation Related to Facial Nerve Damage and Regeneration in Adult Rats." *Experimental Brain Research* 135: 53–65.

Franchi, G. 2001. "Persistence of Vibrissal Motor Representation Following Vibrissal Pad Deafferentation in Adult Rats." *Experimental Brain Research* 137: 180–189.

Gepshtein, S., and M. Kubovy. 2005. "Stability and Change in Perception: Spatial Organization in Temporal Context." *Experimental Brain Research* 160: 487–495.

Gharbawie, O. A., and I. Q. Whishaw. 2006. "Parallel Stages of Learning and Recovery of Skilled Reaching After Motor Cortex Stroke: "Oppositions" Organize Normal and Compensatory Movements." *Behavioural Brain Research* 175: 249–262.

Goldberger, M. E. 1977. "Locomotor Recovery After Unilateral Hindlimb Deafferentation in Cats." *Brain Research* 123: 59–74.

Goodridge, J. P., P. A. Dudchenko, K. A. Worboys, E. J. Golob, and J. S. Taube. 1998. “Cue Control and Head Direction Cells.” *Behavioral Neuroscience* 112: 749–761.

Hartmann, M. J. 2001. “Active Sensing Capabilities of the Rat Whisker System.” *Autonomous Robots* 11: 249–254.

Huntley, G. W. 1997. “Differential Effects of Abnormal Tactile Experience on Shaping Representation Patterns in Developing and Adult Motor Cortex.” *Journal of Neuroscience* 17: 9220–9232.

Inagaki, S., R. Iwata, M. Iwamoto, and T. Imai. 2020. “Widespread Inhibition, Antagonism, and Synergy in Mouse Olfactory Sensory Neurons *In Vivo*.” *Cell Reports* 31: 107814.

Kleinfeld, D., R. W. Berg, and S. M. O'Connor. 1999. “Invited Review Anatomical Loops and Their Electrical Dynamics in Relation to Whisking by Rat.” *Somatosensory & Motor Research* 16: 69–88.

Kurnikova, A., J. D. Moore, S.-M. Liao, M. Deschênes, and D. Kleinfeld. 2017. “Coordination of Orofacial Motor Actions Into Exploratory Behavior by Rat.” *Current Biology* 27: 688–696.

LeCun, Y., Y. Bengio, and G. Hinton. 2015. “Deep Learning.” *Nature* 521: 436–444.

Levy, K. J. 1977. “Pairwise Comparisons Involving Unequal Sample Sizes Associated With Correlations, Proportions or Variances.” *British Journal of Mathematical and Statistical Psychology* 30: 137–139.

Luft, A. R., and M. M. Buitrago. 2005. “Stages of Motor Skill Learning.” *Molecular Neurobiology* 32: 205–216.

Mathis, A., P. Mamidanna, K. M. Cury, et al. 2018. “DeepLabCut: Markerless Pose Estimation of User-Defined Body Parts With Deep Learning.” *Nature Neuroscience* 21: 1281–1289.

Metz, G. A. S., and I. Q. Whishaw. 2000. “Skilled Reaching an Action Pattern: Stability in Rat (*Rattus norvegicus*) Grasping Movements as a Function of Changing Food Pellet Size.” *Behavioural Brain Research* 116: 111–122.

Meyer, M. E., and M. E. Meyer. 1992. “The Effects of Bilateral and Unilateral Vibrissotomy on Behavior Within Aquatic and Terrestrial Environments.” *Physiology & Behavior* 51: 877–880.

Milani, H., H. Steiner, and J. P. Huston. 1989. “Analysis of Recovery From Behavioral Asymmetries Induced by Unilateral Removal of Vibrissae in the Rat.” *Behavioral Neuroscience* 103: 1067–1074.

Mitchinson, B. 2015. “Tactile Attention in the Vibrissal System.” *Scholarpedia* 10: 32361.

Mitchinson, B., R. A. Grant, K. Arkley, V. Rankov, I. Perkon, and T. J. Prescott. 2011. “Active Vibrissal Sensing in Rodents and Marsupials.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 366: 3037–3048.

Mitchinson, B., C. J. Martin, R. A. Grant, and T. J. Prescott. 2007. “Feedback Control in Active Sensing: Rat Exploratory Whisking Is Modulated by Environmental Contact.” *Proceedings: Biological Sciences* 274: 1035–1041.

Mitchinson, B., and T. J. Prescott. 2013. “Whisker Movements Reveal Spatial Attention: A Unified Computational Model of Active Sensing Control in the Rat.” *PLOS Computational Biology* 9: e1003236.

Nguyen, Q.-T., and D. Kleinfeld. 2005. “Positive Feedback in a Brainstem Tactile Sensorimotor Loop.” *Neuron* 45: 447–457.

Nikbakht, N., A. Tafreshiha, D. Zoccolan, and M. E. Diamond. 2018. “Supralinear and Supramodal Integration of Visual and Tactile Signals in Rats: Psychophysics and Neuronal Mechanisms.” *Neuron* 97: 626–639.e8.

Parmiani, P., C. Lucchetti, C. Bonifazzi, and G. Franchi. 2019. “A Kinematic Study of Skilled Reaching Movement in Rat.” *Journal of Neuroscience Methods* 328: 108404.

Parmiani, P., C. Lucchetti, and G. Franchi. 2018. “Whisker and Nose Tactile Sense Guide Rat Behavior in a Skilled Reaching Task.” *Frontiers in Behavioral Neuroscience* 12: 24.

Parmiani, P., C. Lucchetti, R. Viaro, and G. Franchi. 2023. “Long and Short Whiskers Differently Guide Snout/Pellet Interaction in Rat Oral Grasping.” *European Journal of Neuroscience* 58: 2724–2745.

Pellicer-Morata, V., L. Wang, A. de Jongh Curry, J. W. Tsao, and R. S. Waters. 2021. “Structural and Functional Organization of the Lower Jaw Barrel Subfield in Rat Primary Somatosensory Cortex.” *Journal of Comparative Neurology* 529: 1895–1910.

Pfister, J., and W. Zenker. 1984. “The Splenius Capitis Muscle of the Rat, Architecture and Histochemistry, Afferent and Efferent Innervation as Compared With That of the Quadriceps Muscle.” *Anatomy and Embryology* 169: 79–89.

Pouchelon, G., L. Frangeul, F. M. Rijli, and D. Jabaudon. 2012. “Patterning of Pre-Thalamic Somatosensory Pathways.” *European Journal of Neuroscience* 35: 1533–1539.

Prescott, T. J., B. Mitchinson, and R. A. Grant. 2011. “Vibrissal Behavior and Function.” *Scholarpedia* 6: 6642.

Ranade, S., B. Hangya, and A. Kepecs. 2013. “Multiple Modes of Phase Locking Between Sniffing and Whisking During Active Exploration.” *Journal of Neuroscience* 33: 8250–8256.

Raposo, D., J. P. Sheppard, P. R. Schrater, and A. K. Churchland. 2012. “Multisensory Decision-Making in Rats and Humans.” *Journal of Neuroscience* 32: 3726–3735.

Sanes, J. N., S. Suner, J. F. Lando, and J. P. Donoghue. 1988. “Rapid Reorganization of Adult Rat Motor Cortex Somatic Representation Patterns After Motor Nerve Injury.” *Proceedings of the National Academy of Sciences* 85: 2003–2007.

Severson, K. S., and D. H. O'Connor. 2017. “Active Sensing: The Rat's Nose Dances in Step With Whiskers, Head, and Breath.” *Current Biology* 27: R183–R185.

Sherman, D., T. Oram, D. Harel, and E. Ahissar. 2017. “Attention Robustly Gates a Closed-Loop Touch Reflex.” *Current Biology* 27: 1836–1843.e7.

Stein, B. E., T. R. Stanford, and B. A. Rowland. 2014. “Development of Multisensory Integration From the Perspective of the Individual Neuron.” *Nature Reviews Neuroscience* 15: 520–535.

Sutter, C., K. Drewing, and J. Müsseler. 2014. “Multisensory Integration in Action Control.” *Frontiers in Psychology* 5: 544.

Symons, L. A., and R. C. Tees. 1990. “An Examination of the Intramodal and Intermodal Behavioral Consequences of Long-Term Vibrissae Removal in Rats.” *Developmental Psychobiology* 23: 849–867.

Tandon, S., N. Kambi, and N. Jain. 2008. “Overlapping Representations of the Neck and Whiskers in the Rat Motor Cortex Revealed by Mapping at Different Anaesthetic Depths.” *European Journal of Neuroscience* 27: 228–237.

Thé, L., M. L. Wallace, C. H. Chen, E. Chorev, and M. Brecht. 2013. “Structure, Function, and Cortical Representation of the Rat Submandibular Whisker Trident.” *Journal of Neuroscience* 33: 4815–4824.

Towal, R. B., and M. J. Hartmann. 2006. “Right–Left Asymmetries in the Whisking Behavior of Rats Anticipate Head Movements.” *Journal of Neuroscience* 26: 8838–8846.

Vale, R., D. A. Evans, and T. Branco. 2017. “Rapid Spatial Learning Controls Instinctive Defensive Behavior in Mice.” *Current Biology* 27: 1342–1349.

Welker, C., and T. A. Woolsey. 1974. “Structure of Layer IV in the Somatosensory Neocortex of the Rat: Description and Comparison With the Mouse.” *Journal of Comparative Neurology* 158: 437–453.

Welker, W. I. 1964. "Analysis of Sniffing of the Albino Rat 1." *Behaviour* 22: 223–244.

Whishaw, I. Q. 1996. "An Endpoint, Descriptive, and Kinematic Comparison of Skilled Reaching in Mice (*Mus musculus*) With Rats (*Rattus norvegicus*)." *Behavioural Brain Research* 78: 101–111.

Whishaw, I. Q., J. Faraji, J. R. Kuntz, B. Mirza Agha, G. A. S. Metz, and M. H. Mohajerani. 2017. "The Syntactic Organization of Pasta-Eating and the Structure of Reach Movements in the Head-Fixed Mouse." *Scientific Reports* 7: 10987.

Whishaw, I. Q., and J. M. Karl. 2014. "The Contribution of the Reach and the Grasp to Shaping Brain and Behaviour." *Canadian Journal of Experimental Psychology/Revue Canadienne de Psychologie Expérimentale* 68: 223–235.

Whishaw, I. Q., and S. M. Pellis. 1990. "The Structure of Skilled Forelimb Reaching in the Rat: A Proximally Driven Movement With a Single Distal Rotatory Component." *Behavioural Brain Research* 41: 49–59.

Whishaw, I. Q., and J.-A. Tomie. 1989. "Olfaction Directs Skilled Forelimb Reaching in the Rat." *Behavioural Brain Research* 32: 11–21.

Wobbrock, J. O., L. Findlater, D. Gergle, and J. J. Higgins. 2011. "The Aligned Rank Transform for Nonparametric Factorial Analyses Using Only Anova Procedures." In *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems, CHI'11*, 143–146. Association for Computing Machinery.

Wolfe, J., C. Mende, and M. Brecht. 2011. "Social Facial Touch in Rats." *Behavioral Neuroscience* 125: 900–910.

Woolsey, T. A., and H. Van der Loos. 1970. "The Structural Organization of Layer IV in the Somatosensory Region (S I) of Mouse Cerebral Cortex: The Description of a Cortical Field Composed of Discrete Cytoarchitectonic Units." *Brain Research* 17: 205–242.