Brief Report

Tactile Sensory Deprivation Impairs Spatial Behavior in Previsual Rat Pups

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Article

Tactile Sensory Deprivation Impairs Spatial Behavior in Previsual Rat Pups

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Abstract: The vibrotactile system provides essential sensory input for early behavior in previsual rat pups. To examine its role in spatial navigation, we conducted bilateral vibrissectomy or sham procedures on postnatal day (PND) 9–12 in 42 previsual pups (4 litters, balanced groups). Open-field testing on PND 13 showed that while vibrissectomy (VE) did not alter gross locomotor parameters (distance traveled, speed, acceleration, or freezing episodes; all $p > 0.05$), it significantly modified spatial behavior. VE pups explored a smaller percentage of the arena area ($p = 0.01$). We analyzed trajectorial compaction in the central zone (lacking wall tactile support) by smoothing trajectories with virtual coatings scaled to vibrissal length (16 mm). Individual references were generated for each track through linearization and identical smoothing. The compaction ratio, calculated as the ratio between the coated areas of linearized reference and experimental tracks, was significantly higher VE pups compared to sham controls ($p = 0.03$). No significant group difference was observed in path lengths within the central zone. This effect persisted when smoothing scales increased 2–3 fold (32–64 mm radii, approximating body size), but not at smaller scales (2–4 mm). These findings indicate that vibrissal input regulates spatial rather than locomotor components of previsual behavior. The simple open-field measures (area coverage, track compaction) thus serve as sensitive biomarkers for assessing vibrissal function in developing rodents. This study underscores the organizational role of tactile inputs in early spatial navigation and establishes a translatable hallmark for investigating sensory-guided behavior in neurodevelopment.

Keywords: vibrissal system; previsual development; spatial navigation; thigmotaxis; open-field test; rodent model

1. Introduction

Sensory deprivation represents a classical experimental approach in neurobiology [1–4]. The pioneering studies were conducted in the visual system of developing kittens [5], where complete or partial sensory deprivation during critical periods resulted in permanent functional impairments of visual processing. Unlike visual or auditory systems, the rodent somatosensory system permits non-invasive sensory deprivation through whisker trimming. This approach avoids tissue damage while effectively studying deprivation effects without systemic compromise [6].

The precisely organized somatotopic structure of rodents' vibrotactile system provides exceptional opportunities for investigating critical developmental periods and neuronal plasticity [7,8]. The barrel cortex - the primary somatosensory representation of whiskers - derives its name from its distinctive cylindrical functional units. Each barrel receives tactile input exclusively from a single whisker, creating a precise topographic map in somatosensory cortex that mirrors the whisker arrangement on the snout [9–11]. Whisking represents one of the earliest active behavioral patterns for environmental exploration [12], beginning its functional maturation during postnatal days (PND)

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8-9 [13]. Extensive research has characterized both the neuronal mechanisms underlying whisking behavior and the anatomical consequences of whisker removal. Neonatal whisker trimming (vibrissectomy, VE) in rat pups does not prevent barrel formation but leads to expanded excitatory and reduced inhibitory receptive fields [14,15]. The affected cortical regions show area expansion, with spiny stellate neurons exhibiting increased dendritic arborization, greater spine density [6], and modified intracortical connectivity patterns [16] in layer IV of the somatosensory cortex in adulthood.

Despite substantial data on anatomical and electrophysiological consequences of early vibrissectomy in laboratory rodents, simple quantitative measures of immediate behavioral responses to vibrissectomy during spontaneous behavior remain lacking. Vibrissectomy from PND 0 to PND 3 resulted in impaired whisker-specific tactile function at PND30-35 [6] and altered behavioral strategies in gap-crossing tests [17]. Vibrissectomy impaired nipple attachment and huddling behavior when performed at PND 3-5 [12]. Early postnatal whisker removal significantly reduced exploratory activity [18], while prolonged vibrissectomy (PND 9-20) altered defensive behavior development, including threat response patterns [19]. Neonatal whisker ablation caused lasting tactile discrimination deficits, with adult rats showing severe impairments in texture differentiation [20]. Thus, nipple attachment appears to be the earliest behavioral marker sensitive to VE. However, the vibrotactile system in pups should also guide various movements before visual cues become functionally available.

In developing rat pups, locomotor activity emerges before maturation of auditory and visual systems [21]. Initial locomotor actes are driven by olfactory and haptic input. Spatial exploration begins with spontaneous nest departures at PND 10-12 [22]. Immature locomotor patterns (forelimb-driven propulsion and pivoting) mature to quadrupedal locomotion at PND 10-11, with digitigrade gait detectable by PND 14-15 [23–25]. Therefore, prior to visual system activation, the role of the vibrotactile system in spatial exploration can be assessed by experimentally modulating whisker sensory input.

Movement trajectories of organisms exhibit complex characteristics due to measurement noise, micromovements, and inherent path fractality [26–28], raising practical questions about track smoothing and quantification of its nonlinear features. By the end of the second postnatal week, rat pup locomotor patterns include both rapid progressions and lingering phases [29,30], the latter involving environmental scanning (lateral head movements, air sniffing, and small-step displacements). Consequently, sensory deprivation effects are more evident in lingering-related behaviors than during rapid movements, though quantifying such intermittent behaviors remains challenging [31,32].

At PND 13, when rat pups' vibrissae can sweep bilaterally [13] but visual cues remain unavailable, we hypothesized that locomotion patterns at the scale of vibrissal length would be altered in vibrissectomized pups. The hypothesis was checked in the present study.

2. Materials and Methods

2.1. Animals Housing

Outbred Wistar rats were obtained from the Stolbovaya breedery and mated at the Animal Chapter of IHNA&NPh, RAS. Gravid dams were individually housed in standard polycarbonate cages (dimensions: 40 × 60 × 19 cm) with wood saw bedding and additional nesting material (shredded paper towels). The housing facility maintained a 12:12 hr light/dark cycle (lights on at 08.00 A.M), controlled temperature (22 ± 2°C), and humidity (50 ± 10%). Food (standard pellet chow) and water were provided *ad libitum*. Cage beddings were changed twice weekly to ensure hygiene while minimizing stress. Dams were monitored daily for pups' appearance and signs of distress. To reduce disturbance near parturition, cages were left undisturbed from until postnatal day 1 (PND) 1. The birth date was designated PND 0; litters were left intact until PND 9.

2.2. Vibrissectomy

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Before handling the rat pups, the dams were removed from the home cage. Immediately before the procedure, the pups were extracted from the home cage and housed temporarily in a separate cage. On PND 9, pups from each litter were assigned to either an experimental group or a control group. In the experimental group (n=21), all vibrissae on both sides of the snout were gently trimmed to the fur level daily from PND 9 to 12. In the control group (n=21), a sham procedure was performed during the same period by gently applying scissors to the vibrissae bases without cutting. During the procedure, pups were restrained by hand to limit movement and keep warmth. Both procedures (vibrissae trimming and sham) lasted 60–120 seconds per pup. After processing, pups were weighed and marked, then returned to the home cage. The dams were reintroduced afterward.

2.3. Open Field Test

The behavioral testing protocol was performed as described earlier [DOI: 10.1016/j.beproc.2022.104780] with minor modifications. To minimize stress, dams were separated from their litters only after voluntarily leaving the nest. Pups were then allowed to remain undisturbed in their huddles for 30 minutes to achieve stable baseline behavioral states prior to testing. Individual pups were carefully grasped from the periphery of the huddle and transported to the adjacent testing room. Each trial began immediately upon placement of the pup in the center of the arena. The open field apparatus consisted of two detachable pieces: 60cm*60 cm vertical walls, constructed of plywood and painted with black, and replaceable plywood floor plate, covered by black plastic. The design ensured the absence of possible dust and olfactory cues kept in the corners and gaps. Between trials, the arena was cleaned with wet paper towel, 50% ethanol solution and dried with lint-free wipes to eliminate residual olfactory cues that could influence subsequent testing sessions. A video camera was placed 120cm above. The arena had a lighted center (98-102 Lx) and dimmed corners (32-38Lx). A standard spatial orientation of the landed pup was with its head away from the experimenter, directed to the middle of the opposite wall. At the pup's placement, videotracking started and lasted for 120 seconds. After the test, the pups were sexed, weighed, and numbered; the information is summarized in Table 1. The eye status was checked individually. This standardized protocol ensured consistent testing conditions while minimizing potential confounds from handling stress or environmental contamination

2.4. Offline Tracking

2.4.1. ToxTrack

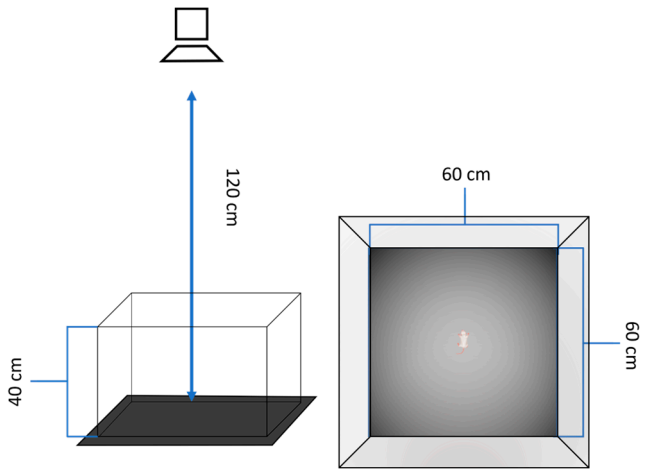

Figure 1. The experimental setup consisted of an open field arena with removable walls constructed from black-painted plywood and a removable plywood floor covered with black plastic. This detachable design prevented the accumulation of potential odor cues in the arena's crevices. A video camera was positioned directly above the center of the arena and connected to a computer in adjacent room.

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All experimental sessions were video-recorded using a digital camera synchronized with a computer located in a next room. Offline tracking of the animals' movements throughout all trials was conducted utilizing the open-source software ToxTrac (v 25.1.1, Umeå, Sweden [33]). The tracking process employed Toxld (Ssi.Rep.)'s proprietary identification algorithm, ToxTrac [34], which calculated the centroid of the white body mass against the black background at 400 ms intervals, thereby digitizing the motion trajectories. Automated measurement included the cumulative distances, and the incidence and duration of freezing episodes.

2.4.2. Trajectory Coating

To quantify the area covered by experimental trajectories, while minimizing graphical noise, we developed a simple method of track coating (Figure 2). Each detected point of the original trajectory was overlaid with a virtual circle (Rv-circle) whose radius matched the mean vibrissal length (Rv) at the given age (PND 13; Rv = 16 mm according to the laboratory data). This coating process smoothed the trajectory with a characteristic spatial scale of Rv (Figure 2). The total area of all superimposed circles was calculated in pixels. The same procedure was applied to the linearized trajectories, where consecutive points maintained their original spacing (see Figure 3 a, f), to construct an individual reference for each experimental trajectory.

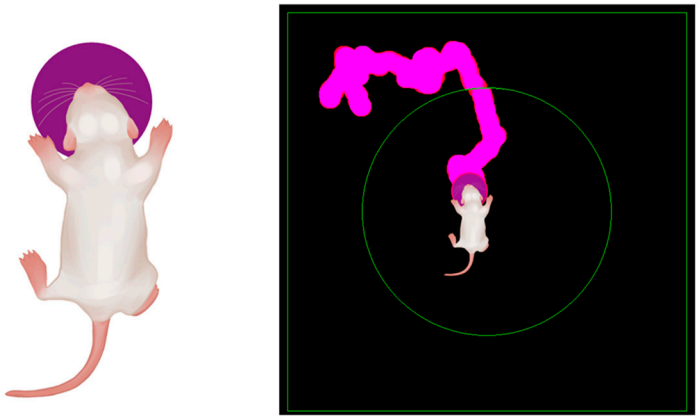

Figure 2. Schematic representation of (left panel) a previsual rat pup with virtual Rv-circle of vibrotactile sensing range, and (right panel) an individual trajectory smoothed through Rv-circle coating at each detection point.

The degree of trajectorial self-attraction (understood as partial or complete returns to the self-track or its vicinity) was quantified using the track compaction ratio:

Cr = S_coated_linearized_track / S_coated_original_track

To differentiate between behaviors with and without haptic support from the arena walls, we established two virtual zones: (1) a central region (20 cm diameter) and (2) the periphery (remaining arena area). All trajectory coating and subsequent analyses focused exclusively on the central zone to isolate locomotor parameters independent of thigmotactic behavior.

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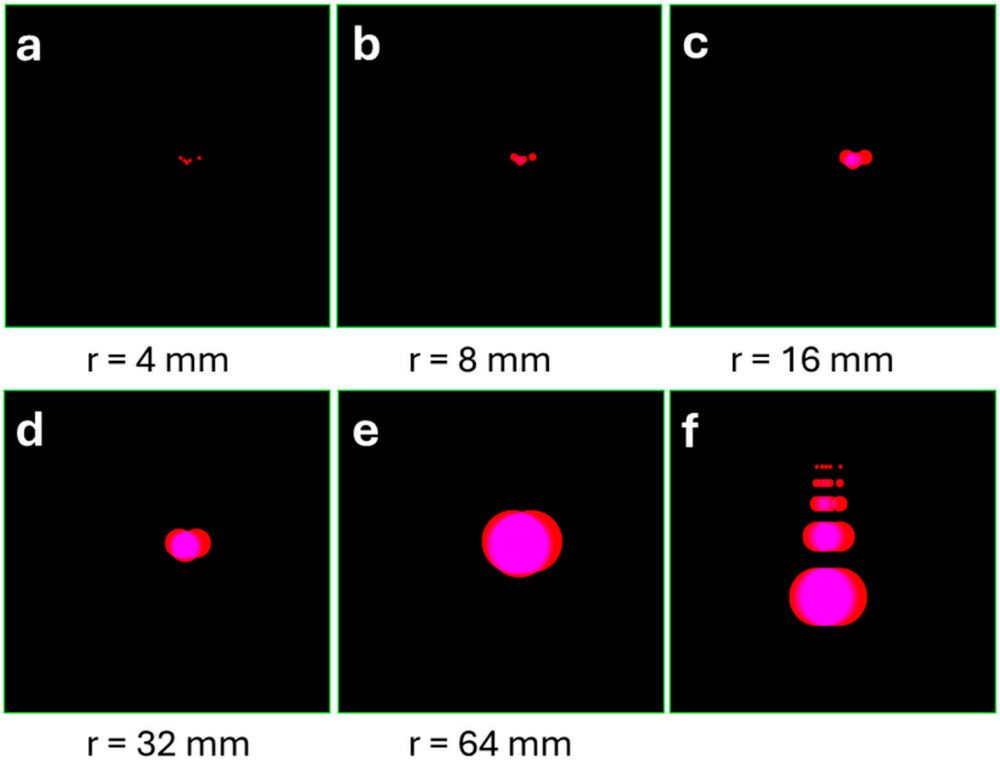

Figure 3. (A-E) Five consecutive points from an original trajectory, each surrounded by R-circles of different radius. (F) The same five sequential trajectory points aligned linearly and coated with corresponding R-circles. Circle radius: 4, 8, 16, 32, 64 mm (given below the panels). To estimate compactness, we calculated the surface area ratio (Cr) between the two coated tracks.

2.4.3. Statistics

The data were analyzed using a general linear model (GLM) ANOVA, with ToxTrac-derived locomotor parameters as dependent variables, group and litter as categorical predictors, and of the session number within a litter as a continuous covariate. It was followed by post-hoc Tukey tests if applicable. Additionally, the Cr values, calculated per pup for a set of coating R (see above), were subjected to the repeated measures ANOVA GLM.

3. Results

3.1. Gross Locomotor Parameters

Inter-litter variability emerged as the primary factor influencing locomotor parameter variation. The experimental manipulation (vibrissectomy) did not significantly alter gross locomotor measures, including total distance traveled, average speed, or freezing duration (Table 1). However, we observed significant between-group differences in exploration rate, with VE pups demonstrating reduced area coverage (Table 1). This parameter showed a tendency to interaction effect (p=0.08), reflecting differential VE impact across litters.

Table 1. Primary locomotor parameters of vibrissectomized (VE) and control (Sham) groups in the open field test. Values represent mean ± SEM. Statistical effects of between-litter variability ("Litter"), vibrissectomy ("VE"), and their interaction ("Litter×VE") were assessed by ANOVA. Significance threshold was set at p=0.05, with 0.05<p<0.10 considered marginal trends. "ns" denotes non-significant effects.

	VE (N=21)	Sham (N=21)	effect	F	p-level

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Total distance, mm	1507±144	1829±189	litter VE litter*VE	F(3,41)=18.8	p<0.001 ns ns
Average speed,mm/s	11.3±1.1	13.9±1.5	litter VE litter*VE	F(3,41)=18.0	p<0.001 ns ns
Average acceleration, mm/s^2	13.5±1.2	15.4±1.4	litter VE litter*VE	F(3,41)=15.6	p<0.001 ns ns
Frozen time, s	12.7±4.6	8.3±2.8	litter VE litter*VE	F(3,41)=6.5	p<0.001 ns ns
Exploration rate, %	0.046±0.004	0.076±0.010	litter VE litter*VE	F(3,41)=7.2 F(3,41)=6.8	p=0.001 p=0.014 tend

The experimental and control groups showed comparable body weights (VE: 22.7 ± 1.5 g vs control: 23.2 ± 2 g; $p>0.10$), confirming that observed behavioral differences were not attributable to developmental or nutritional factors.

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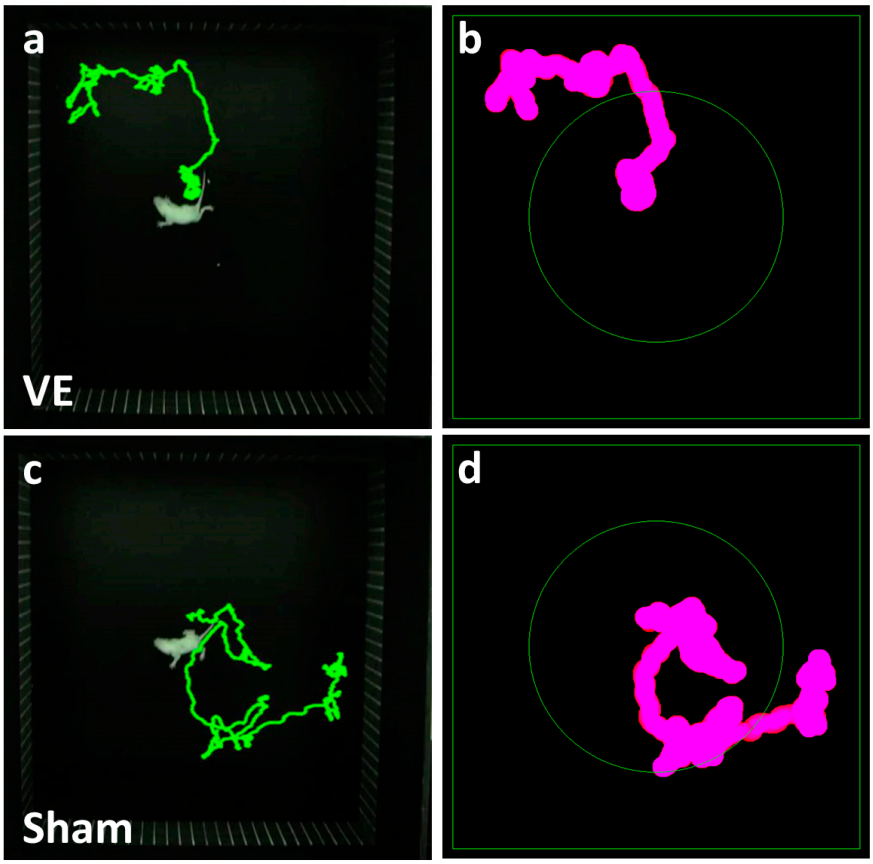

Figure 4. Example trajectories of rat pups from the two groups. (A) Trajectory of an experimental (VE) pup, and (B) the same track smoothed by coating with Rv - circles. (C) Trajectory of a control (sham) pup, and (D) the same track smoothed by coating with RV-circles.

3.2. Compaction of Trajectories Within the Central Zone

The experimental and control pups demonstrated very similar distances travelled within the central zone (VE-group: 1392 ± 690 mm; Sham-group: 1242 ± 723 mm, $p>0.05$). The trajectorial density in the central zone was additionally studied by the developed "Compaction ratio" approach (see Method Section, 2.4.2.).

The sets of the compaction ratios (Cr) were obtained by comparing the covered surface for the original and linearized trajectories, coated with R-circles of radii Rv, and also for Rv/4, Rv/2, 2Rv, 4Rv (Table 2, Figure 3).

The between-litter variability showed the strongest effect on track compaction (Table 2), suggesting potential sensitivity of these parameters to mother-offspring interactions. Beyond this primary familial difference, the vibrissectomy (VE) effect became apparent starting at R=8mm and remained significant for R=16mm, 32mm, and 64mm, with no significant differences observed between these three largest radii conditions (as confirmed by repeated measures ANOVA: R1, $\{F(2,66)= 1.03, p=0.35\}$; R1*VE interaction, $\{F(2,66)= 1.08, p=0.34\}$). This indicates that these compaction ratios remained remarkably stable across the 16-64mm scale. The Cr values show that VE pup trajectories were approximately 10 times more compact than their linearized references, compared to about 6 times for control pups. Notably, the vibrissectomy effect was consistent across litters - in each litter, VE pups developed more compact trajectories than their sham-operated counterparts.

Table 2. Compaction ratio (Cr) values for vibrissectomized (VE) and control (Sham) groups across different coating radii (R, left column). Data presented as mean ± SEM. ANOVA results show effects of between-litter

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variability ("litter"), vibrissectomy (VE), and their interaction (litter*VE). Statistical significance threshold: p=0.05.

R, mm	Cr (VE) mean±SEM	Cr (Sham) mean±SEM	effect	F	p-level	eta-squared
4	5.9 ± 1.2	3.6 ± 0.5	litter VE litter*VE	F(3,41)=6.0	0.002 ns ns	0.35
8	7.9± 1.6	5.6± 0.7	litter VE litter*VE	F(3,41)=6.5 F(1, 41)=4.4	0.001 0.04 ns	0.37 0.11
16	9.6± 1.7	5.8± 0.9	litter VE litter*VE	F(3,41)=8.7 F(1, 41)=5.2	0.0002 0.03 ns	0.44 0.14
32	10.3± 1.5	6.6± 1.0	litter VE litter*VE	F(3,41)=11.6 F(1, 41)=6.6	0.0002 0.02 ns	0.51 0.17
64	9.6± 1.0	6.6± 0.9	litter VE litter*VE	F(3,41)=13.0 F(1, 41)=8.0	0.00001 0.008 ns	0.54 0.19

4. Discussion

We demonstrate that early vibrissectomy significantly alters spontaneous spatial behavior in previsual rat pups, with tactile-deprived animals exhibiting markedly higher track compaction than controls (Tables 1-2, Figure 5). This finding suggests that loss of vibrissal input causes pups to remain closer to their previous paths, while sham controls distribute their movement more broadly across the environment - a phenomenon not previously reported in the literature.

Notably, vibrissectomy did not affect standard locomotor parameters, mirroring observations in epileptic rat models [35,36] and consistent with reports that even spinal cord injury fails to disrupt gross locomotor activity in mouse pups [37]. This remarkable stability of fundamental motor patterns highlights the need for more sensitive behavioral measures.

Several analytical challenges emerge when studying such movement patterns. The inherent complexity of natural trajectories, compounded by measurement artifacts, can mask underlying locomotor strategies [28]. Modern tracking systems (e.g., DeepLabCut, ToxTrac) inevitably combine: (1)Whole-body translations, (2) Pause-associated scanning behaviors [31,32,38], Postural microadjustments [39,40].

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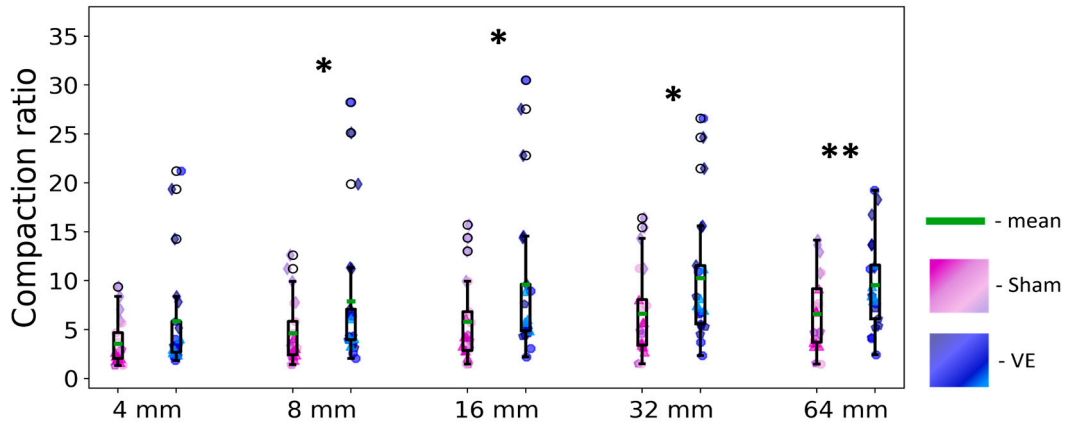

Figure 5. Compaction ratios for the trajectories of the experimental (vibrossectomized) and control (sham) groups, estimated with different coating Rs: 4, 8, 16, 32 and 64 mm (left to right). Note the parameter robustness in a wide range of coating radii.

To address these challenges, we adapted our recently reported space potential method [29], which differentiates pivoting/scanning from directed movement, to create a simplified compaction metric (Cr). This ratio compares R-coated original trajectories to their individual linearized references, which accounting for natural speed. Our prior work established that previsual pups typically show imprecise path retracing, detectable through velocity autocorrelation analysis [29].

The Cr values showed surprising consistency across spatial scales (16-64mm), with VE pups maintaining ratios near 10 versus approximately 6 for controls. This scale-invariant effect, persisting well beyond vibrissal length, implies contributions from additional factors. Presumably, proprioceptive mechanisms related to body size (inter-paw distances: 4-8 cm at this age) may be involved [41–43], though plantar tactile contributions remain poorly understood [44]. Local plantar anesthesia studies could help in future studies.

Alternative interpretations also warrant consideration. Increased compaction in VE pups may reflect not just sensory impairment but also heightened maternal dependence [18], potentially including retrieval anticipation [45]. Thus, while our data clearly establish enhanced path compaction following vibrissectomy (Table 2, Figure 5), the precise mechanistic balance between sensory and affective factors requires further investigation.

These results underscore the essential role of somatosensory input in previsual spatial behavior, with potential relevance for neurodevelopmental disorders featuring sensory integration deficits. The compaction ratio (Cr) proves particularly valuable for detecting moderate yet consistent behavioral consequences of vibrissal deprivation.

5. Conclusions

While sensory-deprived animals maintain unchanged gross locomotor parameters, they exhibit significantly increased trajectorial compaction. The demonstrated sensitivity of the compaction ratio to whisker removal establishes this measure as an easy and robust metric for evaluating vibrotactile function in behaving rodent pups. This approach shows particular promise for translational applications in neurodevelopmental disorder research, where quantitative behavioral markers are urgently needed.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: The gross locomotor parameters in individual pups. Table 2S. The compaction ratios in individual pups. Table 3S: The script details. Figures 1S-8S: origimal trajetories of VE and sham pups of each litter.

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Data Availability Statement: All the original trajectories are published as Supplementary materials to this manuscript

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Cr	compaction ratio
PND	postnatal day
mm	millimeter
R	radius
s	second
VE	vibrissectomy

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