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Effect of Spontaneous Partial Sensory Deprivation on the Behavior of Male C57BL/6N Mice

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The absence of whiskers, which is associated with the features of within-group interactions in animals, can be an important factor affecting the results of tests assessing their behavior. The effects of spontaneous partial sensory deprivation due to barbering was studied in male C57Bl/6N mice using a series of behavioral tests. The results indicate that the behavior of mice without whiskers cannot be classified as anxious-depressive, though there were significant differences from the behavior of control animals in the tube, open field, social interaction, and forced swimming tests. Thus, the results provide evidence that when running behavioral tests, the state of the whiskers should be assessed before inclusion of the animals into experimental groups and/or spontaneous barbering should be considered in the statistical analysis of the data from these tests.

Keywords: barbering, male C57Bl/6N mice, tube test, open field test, social interaction test, forced swimming test.

Loss of the whiskers and fur associated with dermatological diseases and animals' behavior is seen periodically in different mammal species [21]. This phenomenon, first described in laboratory mice as a result of whisker-eating [16] was further termed hair-nibbling and whisker-trimming [19, 23], the barbering effect [9], overgrooming, trichophagy, dewhiskering, whisker/hair plucking/pulling, and even the Dalila effect [22].

Barbering behavior, which is quite widespread in laboratory animals, can be self-directed (self-barbering) or directed to a littermate (allobarbering or heterobarbering). Nibbling of the animal's own fur in the abdominal area, genitals, or the internal surfaces of the hindlimbs [15] is seen more often in mice kept in conditions of social isolation [21]. Heterobarbering can also be seen in single-sex groups of mice (mostly in females) [21] as well as in different-sex pairs, where the barber (the animal nibbling the fur) may be either the male or the female [22]. The barber living in a group of littermates nibbles whiskers and the fur around the eyes and on the head or back, and more rarely in other locations (Fig. 1), all mice receiving allobarbering showing similar topography [15]. Experimental studies have demonstrated that all animals in the group maintain this anomalous behavior. Thus, keeping a barber on the other side of a partition preventing it from entering the recipients' territory but allowing tactile contact when littermates approach the grid voluntarily did not lead to any increase in alopecia in these animals, while the fur on the nibbled area of recipients' bodies recovered when there was no tactile contact with the barber [25].

The principles of behavior leading to dermatopathy [24] thus far remain to be understood. It was initially proposed that nibbling of the whiskers and fur was an indicator of dominance, as signs of "shaving" started to appear in mice during the process of despotic establishment of the hierarchy [15], while voluntary establishment of a submissive individual to grooming is something different, a means of minimizing the aggression of the dominant animal [2]. A genetic predisposition to this behavioral reaction was seen, though the social environment could also promote the occurrence of barbering [12]. "Shaving" is most widespread in C57BL/6 (B6) and A2G mice [19, 22, 23]. This has been noted as normal in animal houses for laboratory mice [17], while on

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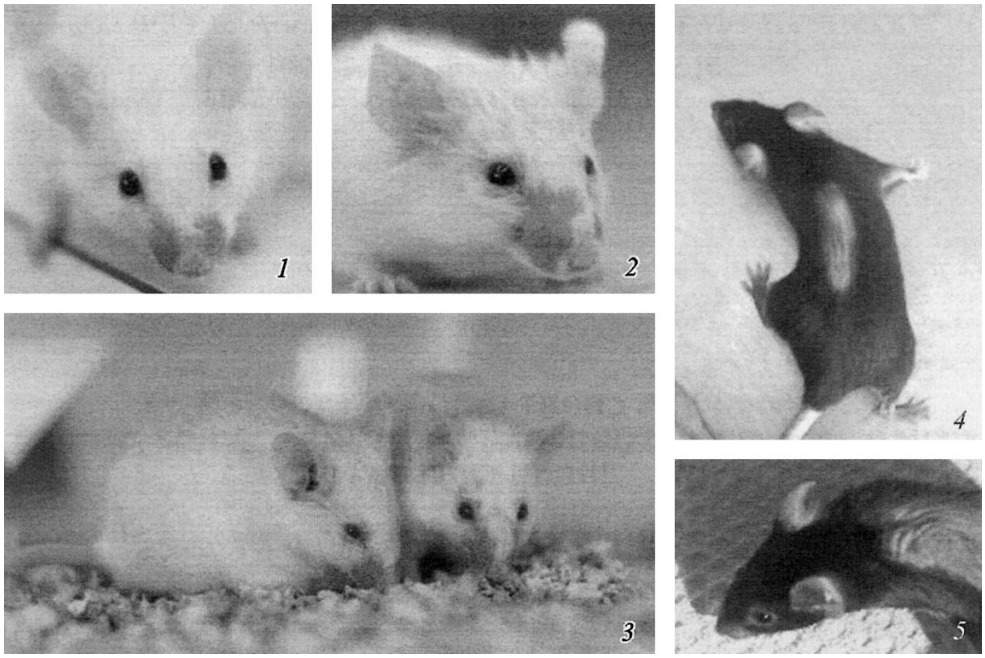

Fig. 1. External appearance of mice treated by a barber. *1–3*) Male outbred Swiss mice (from Rappolovo, Russia); *4–5*) male C57Bl/6N mice (from Pushchino, Russia).

evaluation of the state of health, alopecia arising as a result of barbering is not regarded as pathology [8]. Mice among which barbering occurs are also known to retain this tendency even when nurtured by normal adoptive parents, while offspring reared by adoptive barbering parents displayed fur-shaving behavior. It is also recognized that barbering is an idiosyncratic reaction which is determined or precipitated by environmental factors including: poverty of keeping conditions (lack of shelter, best-building materials, etc.), social factors (overcrowding), diet, etc. It has been suggested that this may be a form of adaptation of animals to inadequate keeping conditions [25], as enrichment of the environment decreases the severity of fur-shaving behavior, albeit not eliminating it completely [2, 9]. Worsening of anomalous behavior in groups of mice may occur when the diet has a low protein content and a high (0.9%) L-tryptophan content, which alters serotonin metabolism [11]. The experimental data provide evidence that this anomalous behavior affects both the functioning of the brains of both the barber [14] and its victims [22]. Thus, barbering may be an indicator of stress and constitutes a compensatory mechanism analogous to the expression of trichotillomania (compulsive pulling out of hair) in humans [15]. Thus, behavioral dermatology spontaneously arising in subpopulations of laboratory mice is regarded as a model of trichotillomania, with a high degree of external validity [13, 24]. Barbering is also regarded as a model of obsessive compulsive spectrum disorders [15], though the subsequently observed absence of limbic biomarkers refutes the constructive validity of this model [13].

Mice living in a group with a barber generally lose primarily the fur on the whisker pads. Remaining without a "moustache," the animals are thus subjected to partial sensory deprivation. This may affect their behavior, especially in conditions of novelty, as the whiskers are the mechanical part of the vibrotactile analyzer, which plays the key role in organizing adaptive behavior [7, 10]. The whiskers are extremely important for close-range orientation, as they allow the animal to obtain information abut what is immediately in front of the snout, i.e., the zone which, because of the lateral position of the eyes, is not accessible to visual perception [5]. The whiskers provide for primary investigations of objects and establishment of the most important characteristics – shape and surface texture – which determine subsequent manipulations of objects [4]. Removal of the whiskers in rats is regarded as a model of anxiety [3], so it can be suggested that spontaneous dewhiskering of mice due to barbering may promote increases in their levels of anxiety.

The aims of the present work were to undertake a comparative analysis of the individual and social behavior of male mice without and with moustaches to assess the potential of using spontaneous partial sensory deprivation as a model of a depression-like state and to determine the need for using spontaneous dewhiskering as an exclusion criterion for experiments on mice.

Methods. Experiments were performed on adult male C57BL/6N mice (from the Pushchino laboratory animal supplier, Russia). Mice were kept in groups in standard TIIIH polysulfone cages (Tecniplast, Italy) with free access to a

TABLE 1. Tests Used in the Experiments

Tests	Experimental days														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Open field	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tube	–	–	–	±	±	±	+	+	–	–	+	+	+	+	–
Paired interaction	–	–	–	–	–	–	+	+	–	–	+	+	+	+	–
Forced swimming	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+

Note. ± – training to individual passage through tube (three trials per day).

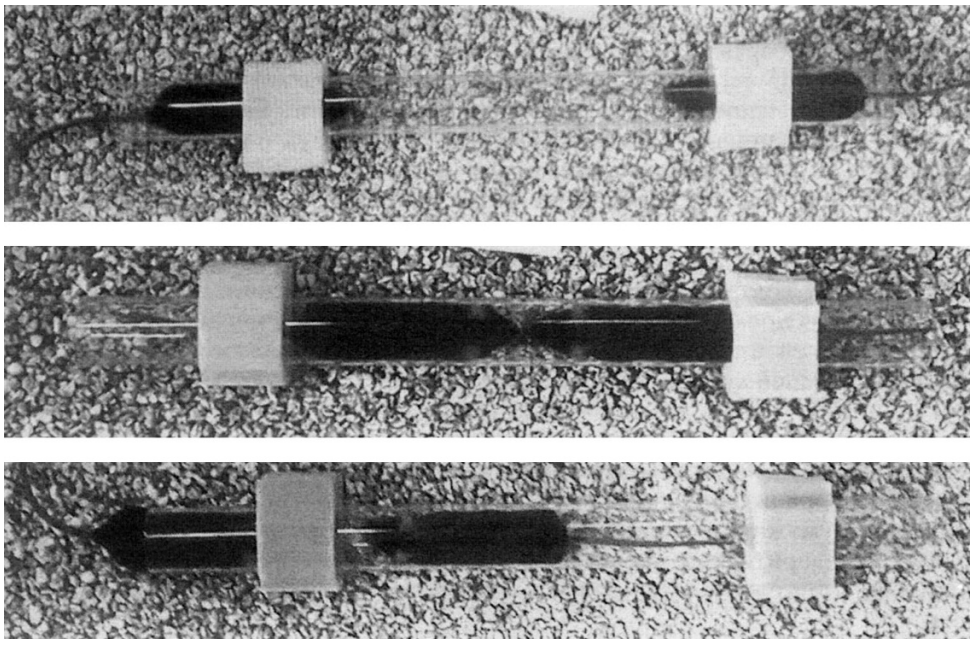

Fig. 2. Tube test assessing dominance behavior in mice

full diet of combined feed (Laboratorkorm, Russia) and filtered (Akvafor, Russia) tap water. The animal keeping location adhered to a light cycle of 12 h light and 12 h dark, with the light turned on at 09:00), a temperature of 20–24°C, and a relative humidity of 50 ± 20%. The litter material consisted of granules made from corn cobs (ZKK Golden Cob, Russia). Litter was changed twice a week. Group 1 consisted of seven mice including a barber which mainly shaved the whisker pads; group 2 consisted of six animals with intact fur. All experiments were performed in compliance with the requirements of the Guidelines for the Use of Laboratory Animals for Scientific and Educational Purposes of the Pavlov First St. Petersburg State Medical University [1].

Experiments were performed over 15 days, analyzing the behavior of mice in a series of tests (see Table 1): the open field test supplemented by the presence of unfamiliar objects; social interaction tests, i.e., the tube displacement test and the paired interaction test on neutral territory, and the forced swimming test. The behavior of the mice was recorded using a digital camera, and video recordings were used to document the sequences and durations of different acts and postures using the Ethograph program (version 2.07, RITEK, St. Petersburg, Russia).

Open field test. This test assessed movement and exploratory activity in an anxiogenic novelty situation. The open field apparatus consisted of a square arena of size 50 × 50 cm with walls 35 cm high made of opaque polycarbonate. The floor of the arena was divided into nine identical sectors with different attractivenesses for mice leading a crepuscular lifestyle and with marked thygmotaxsis. Unfamiliar glass objects with different sizes, shapes, and surface structures were placed in two of the four corner sections (along the diagonals). Mice were placed in the central square of the arena and their behavior was then followed using a video camera (Sony HDR-CX155E) for 5 min. After the test, the numbers of deposited fecal boluses (the index of emotionality) were counted and the apparatus was washed with hydrogen peroxide solution to eliminate odors. Then, analyz-

ing the video recordings, the time spent in each zone of the open field (the center, walls, and corners) was determined, along with movement (runs) and exploratory (sniffing and tactile contact with objects, rearing onto the hindpaws) activity and other elements of individual behavior (self-grooming, scratching, etc.).

The tube test [18]. The experimental apparatus consisted of a tube (3 cm in diameter, 36 cm long) made of transparent Plexiglas (Fig. 2), which was placed on the litter in a TIV cage (Tecniplast, Italy). During the two days preceding the test, each mouse was individually trained to pass through the tube, using three trials. Tests were then performed daily, using pairs such that by six days each animal of the "moustached" group encountered each mouse of the "non-moustached" group (6 × 6). Mice were simultaneously launched into opposite ends of the tube and the time from placing the animals in positive to encountering an opponent from the unfamiliar litter was noted. At 5 min after the test, each pair of mice was given the opportunity to interact freely on neutral territory (the paired interaction test). After each test, the tube was washed thoroughly and wiped dry with paper towels.

The paired interaction test. At 5 min after the tube test, the same pair of mice was simultaneously placed in the opposite corners of a transparent box of size 35 × 25 × 35 cm (length × width × height) with fresh litter material and behavior was recorded with a digital camera for 5 min. Each video recording was evaluated twice, recording the sequence and durations of 40 elements of the social and individual behavior of each animal separately: movement activity (runs, rearings onto the hindpaws), investigations of partner (sniffing the body, nose, and anogenital region, allogrooming), and agonistic (threats, attacks, defensive rearings, etc.), comfort (grooming, scratching, sniffing), and feeding behaviors.

The forced swimming test. This test assesses the acute (situational) behavioral reactions of mice to unavoidable aversive stimulation. Mice were placed accurately in individual glass cylinders (diameter 12 cm, height 20 cm) filled with water (24°C) to a depth of 15 cm and separated from each other by opaque partitions. Test duration was 6 min. After the test, animals were accurately removed from the cylinder, wiped with paper towels, and placed in a cage with clean litter and paper napkins. The water in the cylinder was changed after each mouse, after having counted the number of boluses left in the water; the cylinders were washed thoroughly to eliminate the odor of the preceding animal.

Video recordings were processed to evaluate the frequencies and durations of the following groups of behavioral elements: 1) immobility ("drifting" in the vertical or horizontal position, characterized by the absence of paw movement apart from movements needed to maintain the head above the water surface – floating); 2) orientation (swimming – movement by moving all the limbs and the tail; paddling – movement by making rhythmic hindlimb movements); 3) active escape (clambering up the wall – intensive movement of all limbs with the paws rising above the surface of the water – climbing); 4) comfort behavior, providing evidence that the animal was experiencing discomfort (shaking of the head to remove water from the ears and nose – shaking; wiping the snout to remove water from the eyes – washing).

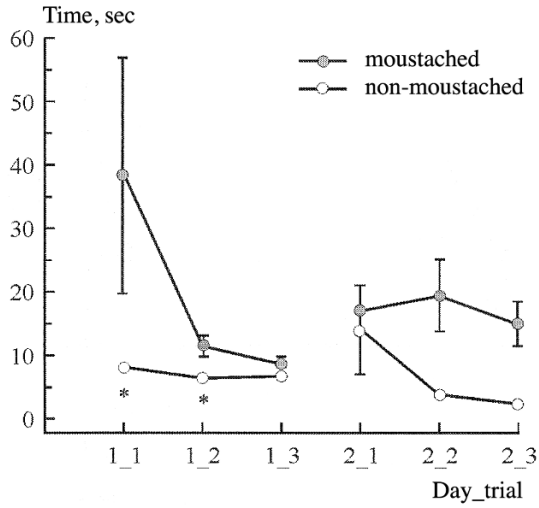

Fig. 3. Rate of passage through tube. Data are shown as $M \pm m$ mean time spent in tube on sequential passages through it. *Significant differences from values in moustached mice, $p < 0.05$ (Bonferroni test).

Data were processed in SigmaPlot version 12.5 (Systat Software Inc., Chicago, USA). Normal distributions were confirmed using the Shapiro–Wilks test. Significant differences ($p < 0.05$) were identified using the *t* test or the Mann–Whitney test for paired comparisons, along with analysis of variance (ANOVA) for absolute magnitudes or their ranked values followed by between-group comparisons (the Bonferroni test).

Results. *Open field test*. The times spent in the different parts of the open field apparatus and the durations of the behavioral elements recorded were not different between moustached and non-moustached mice, though there were differences in the structure of their interactions with glass objects. The total durations of sniffing objects and tactile contact with them were similar ($p = 0.82$, Mann–Whitney test), though the proportion of sniffing was significantly ($F_{1,10} = 55.1, p < 0.001$) less in mice lacking whiskers (about 25% as compared with 82% in moustached mice). The surface characteristics of the objects affected the structure of the interactions with them ($F_{1,19} = 0.05, p = 0.83$). Differences in the emotional reacting of moustached and non-moustached mice to novelty, assessed in terms of the number of fecal boluses, also failed to reach the level of significance. Overall, the proportion of animals leaving boluses was smaller in the group of non-moustached mice.

The tube test. During training of mice to pass through the tube, the rate of movement of non-moustached mice was

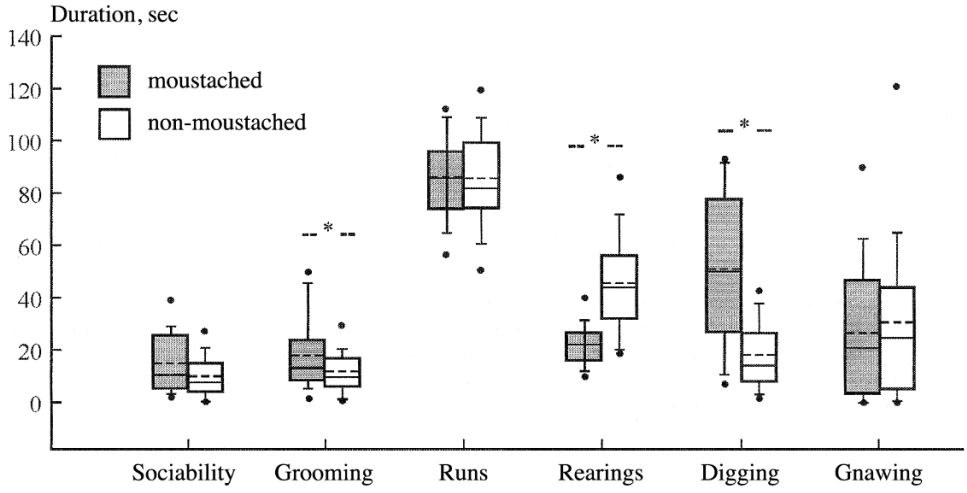

Fig. 4. Behavior of mice in paired interaction test. Distributions of durations of behavioral elements are shown as block and whisker diagrams – the center of the block shows the mean (dotted line), the median (continuous line, the 25% and 75% quartiles (upper and lower ends of boxes); the 10th and 90th percentiles are shown by the upper and lower whiskers) and the 5th and 95th percentiles by dots. $n = 6$ for each group. $^*p < 0.05$ (t test or Mann–Whitney test).

greater (Fig. 3) on both the first and second training days ($F_{1,10} = 25.9$, $p < 0.001$ on day 1 and $F_{1,10} = 8.0$, $p < 0.05$ on day 2). The proportion of victories (pushing out of the tube) on placing pairs in tubes was greater in moustached mice (61%, compared with 39%, a total of six duels), though the difference did not reach level of significance, perhaps because of too small a cohort of experimental animals.

The paired interaction test. The behavior of moustached and non-moustached mice in the paired interaction test differed in terms of a number of measures (Fig. 4). Comparison of the absolute durations of behavioral elements or their complexes was performed using mixed effects analysis of variance, where the fixed factor was the group of animals (two levels – moustached and non-moustached) and the random factor was the sequence number of the test (six levels). No behavioral indicator showed a significant influence for the random factor or its interaction with the fixed factor. The duration of investigating the partner was shorter in non-moustached mice, though the difference did not reach statistical significance ($F_{1,60} = 4.87$, $p = 0.079$). Non-moustached mice spent more time in vertical rearings ($F_{1,60} = 43.59$, $p < 0.001$) and less time digging and strewing the litter material ($F_{1,60} = 61.10$, $p < 0.001$) and cleaning their own bodies ($F_{1,60} = 10.83$, $p < 0.05$).

The forced swimming test. In the forced swimming test, the behavior of moustached and non-moustached mice differed mainly during the first 2 min of the test (Fig. 5). Two-factor repeat measures analysis of variance (the between-subject factor was the experimental group and the within-subject factor was one-minute intervals) did not identify any influence of the group factor on the duration of drifting ($F_{1,10} = 1.3$, $p = 0.28$), though the interaction of these factors ($F_{5,50} = 2.6$, $p < 0.05$) had a significant influence. Group assignment was significantly influenced by the durations of paddling and clambering ($F_{1,10} = 5.2$, $p < 0.05$ and $F_{1,10} = 6.7$, $p < 0.05$, respectively), the interaction of these factors being significant only for the second of these parameters ($F_{5,50} = 6.5$, $p = 0.05$). The number of boluses left in the water was somewhat greater for moustached mice, though the difference did not reach the level of statistical significance ($p = 0.18$, Mann–Whitney test)

Discussion. Despite the fact that the behavioral dermatopathy phenomenon in mice was first described more than 60 years ago [16], there are relatively few experimental studies addressing the behavior of mice in conditions of spontaneous partial sensory deprivation. The differences in the individual and social behavior of moustached and non-moustached mice seen in the present studies point primarily to the importance of the sensory influx for the expression of behavior, though it does not provide evidence of a depression-like state or an increase in the level of anxiety in animals spontaneously losing their whiskers. Thus, the movement activity of mice in the open field was independent of whether or not they had whiskers, while active avoidance of aversive conditions in the forced swimming test by moustached mice in the first minutes of the test was even greater than in controls. None of the tests evaluating emotionality in terms of the numbers of fecal boluses left in the experimental apparatus identified any significant differences, though this parameter was always somewhat smaller in non-moustached mice. Decreased defecation has previously been noted in vibrissotomized rat pups the open field test [6], suggesting that they have low levels of emotional reactivity, preventing the formation of active defensive behavior.

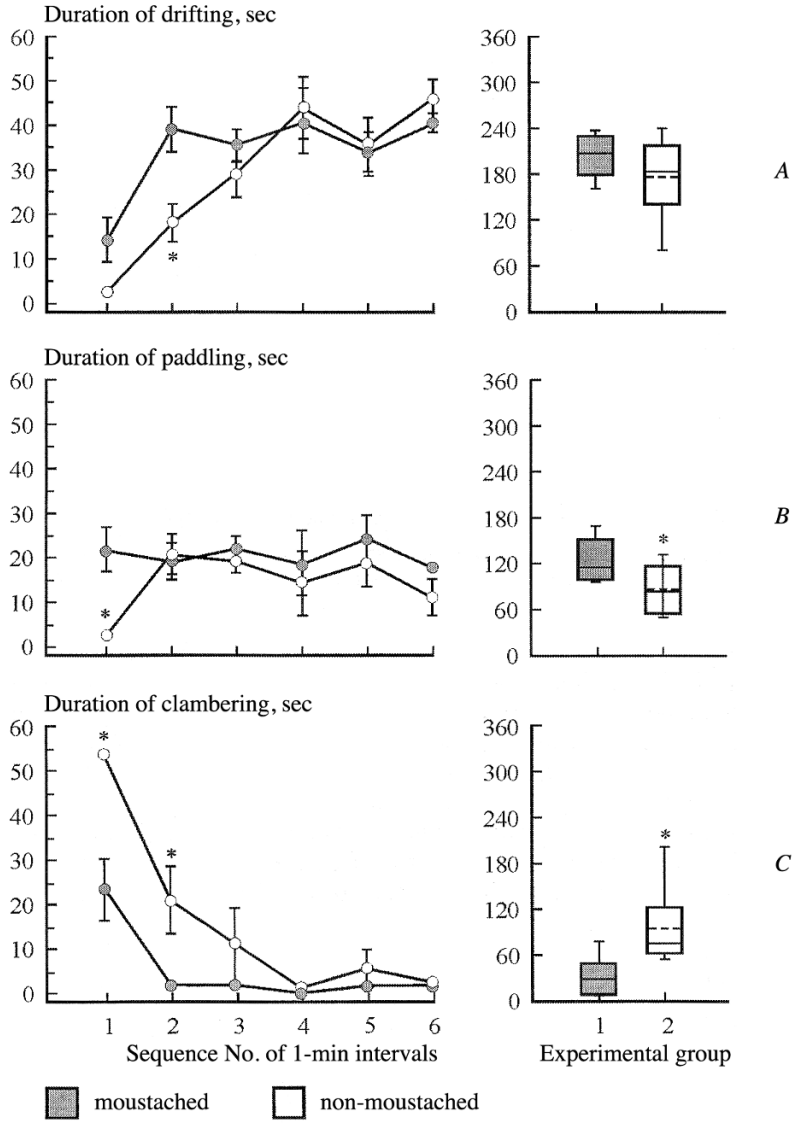

Fig. 5. Behavior of mice in the forced swimming test: immobility (*A*), movement (*B*), and escape (*C*). Left: $M \pm m$ durations of behavioral elements for each minute of the test. Right: distribution of durations of behavioral elements throughout the test (6 min) as block and whisker diagrams – the center of the block shows the mean (dotted line), the median (continuous line, the 25 and 75% quartiles (upper and lower ends of boxes); the 10th and 90th percentiles are shown by the upper and lower whiskers. $n = 6$ for each group.*$p < 0.05$ (Bonferroni test).

Thus, partial sensory deprivation of mice associated with the behavior of a barber present in the cage led to changes in exploratory behavior in the context of novelty, whether this was in an open or closed space or an atmospheric or aqueous environment. These changes are associated with the fact that non-moustached animals are unable to carry out tactile investigation of new objects, which in all probability leads to changes in the structure of familiarization with novel objects, higher rates of passage through the tube, and shorter durations of orientational behavior in the water-filled reservoir. The results of previous experimental studies also provide evidence that the whiskers are involved in maintaining the animal's head above the surface of the water [20] and determine the rate of warming of mice immersed in water with a load attached to the tail [26].

Furthermore, the results obtained here have applied value for planning experimental studies using mice. Reports of results from experimental studies do not generally describe the inclusion criteria of animals for experiments, while statistical processing of experimental data does not identify the spontaneous dewhiskering factor as a random factor and does not assess its contribution to the results obtained. Considering the fact that this phenomenon is quite widespread in a number of mouse strains [2], possible distortion can be suggested primarily of behavioral experimental data because of the use of animals with sensory deprivation.

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