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The role of whiskers in compensation of visual deficit in a mouse model of retinal degeneration

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HIGHLIGHTS

- In the seeing mice, the removal of the whiskers had no effect.
- In the blind mice, whiskers are essential for the compensation of the visual deficit.
- Both chronic and acute tactile deprivation induced anxiety-like behaviour.
- A combination of blindness and chronic tactile deprivation enhances hearing.

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ABSTRACT

Sensory deprivation in one modality can enhance the development of the remaining modalities via mechanisms of synaptic plasticity. Mice of the C3H strain suffer from RD1 retinal degeneration that leads to visual impairment at weaning age. We examined a role of whiskers in compensation of the visual deficit. In order to differentiate the contribution of the whiskers from other mechanisms that can take part in the compensation, we investigated the effect of both chronic and acute tactile deprivation. Three-month-old mice were used. We examined motor skills (rotarod, beam walking test), gait control (CatWalk system), spontaneous motor activity (open field) and CNS excitability to an acoustic stimulus for assessment of compensatory changes in auditory system (audiogenic epilepsy). In the sighted mice, the only effect was a decline in their rotarod test performance after acute whisker removal. In the blind animals, chronic tactile deprivation caused changes in their gait and impaired the performance in motor tests. Some other compensatory mechanisms were involved but the whiskers are essential for the compensation as it emerged from more marked change of gait and the worsening of the motor performance after the acute whisker removal. Both chronic and acute tactile deprivation induced anxiety-like behaviour. Only a combination of blindness and chronic tactile deprivation led to an increased sense of hearing.

1. Introduction

Sensory deprivation in one modality can enhance the development of the remaining modalities. This cross-modal plasticity has been documented both in animals and humans deprived of a particular sensory modality. A loss of vision is compensated by an increased perceptiveness in the somatosensory, auditory and olfactory systems [6,15,17]. In many animals, the most important means of tactile orientation are the mystacial vibrissae. The whiskers also play an important role in complex animal behaviour [12]. As a consequence of visual deprivation from birth, facial vibrissae exhibit supernormal growth in cats and mice followed by cortical rearrangement [14].

Retinitis pigmentosa is one of a group of inherited human diseases which photoreceptor degeneration leads to a visual loss and to eventual blindness in. It is a major cause of progressive retinal disease worldwide [9]. There are sixteen naturally occurring mouse mutants that manifest degeneration of photoreceptors in the retina with the preservation of all other retinal cell types.

In mice of the C3H strain, retinal degeneration similar to a form of retinitis pigmentosa in humans is determined by a homozygous combination of degeneration RD1 mutation (*Pde6b*^{rd1}) which causes blindness at weaning age [5]. While an almost complete absence of the photoreceptors negatively affects visual orientation [18], circadian responses to light are the same as in mice with normal retinas [7].

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In our previous experiments using C3H mice, we observed that retinal degeneration does not influence gait parameters and behaviour of the animals in the open field. We concluded that whiskers may play a role in this finding and this suggested to us the need to perform the present study.

The aim of the present study was to evaluate the role of facial vibrissae as compensation for the visual deficit caused by the retinal degeneration. To differentiate the contribution of this mechanism from other ones which can take part in the process of the compensation, we investigated the effect of both chronic and acute deprivation of the tactile stimuli mediated via whiskers. We examined gait control and spontaneous activity, whereby we observed some effect and parameters in which we anticipated possible role of the whiskers: motor skills and the CNS excitability to an acoustic stimulus for evaluation of compensatory changes in auditory system.

2. Materials and methods

2.1. Animals

Adult mice of C3H strain were used ($n = 71$). The mean age of animals was 90 ± 2 days. The mice were bred under standard conditions with 12:12 h light/dark cycle (light phase 6 a.m. to 6 p.m.), temperature 22–24 °C. They were housed in plastic cages (22 cm × 25 cm, 14 cm high) with a metal mesh cover, with 2–5 mice/cage. Food and water were available *ad libitum*. The mice were obtained at the Department of Pathophysiology, Faculty of Medicine in Pilsen. All the experiments reported here were conducted in full compliance with the EU guidelines for scientific experimentation on animals and with the permission of the Ethical Commission of the Faculty of Medicine in Pilsen. All efforts were made to minimize the number of animals used and their suffering.

2.2. Design of the experiment

Six groups of mice were constituted according to the presence or absence of retinal degeneration and the preservation or removal of the whiskers: 1 – intact sight/preserved whiskers (Rd− Wh); 2 – intact sight/long-term tactile deprivation (Rd− Wh/Lt); 3 – intact sight/acute tactile deprivation (Rd− Wh/Ac); 4 – retinal degeneration/preserved whiskers (Rd+ Wh); 5 – retinal degeneration/long-term tactile deprivation (Rd+ Wh/Lt); 6 – retinal degeneration/acute tactile deprivation (Rd+ Wh/Ac). The animals in the individual experimental groups numbered between 11 and 13. Since our previous experiments revealed no differences between males and females, mice of both sexes were used in equal number per group.

In the group of mice with chronic tactile deprivation, the whiskers were first cut at P10 when they started to be apparent and then had been cut regularly once a week up to the start of the experiments. In case of acute tactile deprivation, whiskers were cut one day prior to the experiments. At the age of three months, experiments started with the motor tests and an open field test. On the next day, their gait was examined and CNS excitability was tested. Immediately after the latter, the mice were sacrificed by thiopental overdosing and their retinas were processed for histologic examination.

2.3. Experimental procedures

Motor coordination was examined by means of the two standard methods: the rotarod and the beam-walking test. In the rotarod test, the mice were placed on a rotating cylinder with a 4 cm diameter. The cylinder was divided into 6.5-cm segments with plastic coils (diameter of 22 cm, concentric with the cylinder). The coils delimited spaces for individual mice. The speed of the rotation was 4 turns/min. In the beam walking test, the animals were put in the middle of the wooden beam (100 cm long, 8 cm diameter) 50 cm above a table covered with a soft pillow. In both methods, the mice were subjected to four trials. Between the trials, there were 5-min resting intervals in their home cage. Fall latencies were measured. If the mouse reached the latency of 120 s, the trial was stopped. We calculated mean fall latencies of the four trials for individual animals.

The gait parameters were tested using the CatWalk system (Noldus Information Technology BV, Wageningen, The Netherlands). The animals were put in a corridor (85 cm long and 8.5 cm wide) and allowed to move freely across the walkway. For each mouse, five tracks with a straight continuous cross over the walkway were acquired and analyzed, and then the values from the individual tracks were averaged. The following parameters were evaluated: walking speed (in mm/s), regularity index (% of regular step patterns), paw angle (angle in degrees formed between the long axis of the paw and the line given by the walking direction), stride length (in mm), stand (duration of the standing phase in s), swing (duration of the swing phase in s), swing speed (in m/s), base of support (distance in mm between the limb pairs in the girdle), and finally support (combination of paws simultaneously in contact with the walkway in % of walking time). Paw angle, stride length, stand, swing and swing speed values were all evaluated separately for both fore and hind paws while the left and right paw values were averaged. For a complete description of these parameters, the reader is referred to the paper by Hamers et al. [8].

Spontaneous motor activity was examined in the open field. The mouse was placed into the centre of a square arena (40 cm × 40 cm, height of the walls 40 cm) and was allowed to move freely for 10 min. The trajectory of the animals was registered and processed with the EthoVision system (Noldus Information Technology BV, The Netherlands). The trajectory length and relative time spent in the central zone were evaluated.

The method of audiogenic epilepsy was used to test CNS excitability. The mice were put into a plastic box (58 cm × 36 cm × 19 cm) with a transparent cover and exposed to a sound stimulus of 90 dB for a time period of 60 s. The reactions of the animals to the stimulus were graded in a 5° scale according to Cendelín and Vožeh [4]: 1 – no reaction; 2 – running; 3 – running and jumping; 4 – tonic–clonic convulsions; 5 – convulsions followed by death of the animal.

The presence or absence of retinal degeneration was histologically examined using hematoxylin–eosin stained 16 μm cryostat sections.

2.4. Statistical evaluation

For all experiments, we compared results of sighted versus blind mice with the preserved whiskers. In both sighted and blind animal subgroups, we compared the mice with the preserved whiskers versus the mice with either the long-term or acute tactile deprivation. Because the data did not show normal distribution as indicated by the Kolmogorov–Smirnov test, for all parameters and all experimental groups, non-parametric the Mann–Whitney test followed by Bonferroni correction was used. In all cases, $p < 0.05$ was considered statistically significant. The results in the text and figures are expressed as the means ± SEM. GraphPad InStat 3.06 (San Diego, CA, USA) software was used.

3. Results

There were no differences between the sighted and blind animals with the intact whiskers in all performed tests. Also no differences between male and female mice were observed.