Functional Consequences of Retinal Degeneration in Spatial Orientation in C3H Wild Type and Lurcher Mutant Mice

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Abstract: Lurcher mutant mice represent a model of genetically determined olivocerebellar degeneration. In the C3H strain there is also hereditary retinal degeneration. The aim of this work was to assess, whether the retinal degeneration influences spatial orientation and results of the spatial learning tasks. Two experiments in the Morris water maze were arranged. First, mice learned to find a platform position, which was linked to two labels on the periphery of the maze. In the second experiment the platform was removed and swimming velocity and preference of central or peripheral zone of the maze were assessed. Presence of the retinal degeneration was detected histologically. Both Lurcher mutant and wild type mice that exhibited long latencies in the first experiment were affected with the retinal degeneration, while animals that performed the trial well, had normal retina. Swimming velocity was not changed substantially. The maze exploration strategy was different in mice with and without the retinal degeneration.

Key words: Lurcher mutant mice – Cerebellar degeneration – Retinal degeneration – Spatial orientation – Morris water maze

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Introduction

Lurcher mutant mice represent a natural model of genetically determined olivocerebellar degeneration [1]. It is caused by a mutation in the δ2 glutamate receptor gene. Heterozygous individuals (+/Lc) suffer from postnatal loss of Purkinje cells, which die by excitotoxic apoptosis [2] and secondary decrease of cerebellar granule cells and inferior olivary neurons. As a consequence of the loss of Purkinje cells the cerebellar ataxia develops in Lurcher mutants at the end of the second postnatal week and they also have deteriorated some cognitive functions, including spatial learning abilities [3, 4]. Wild type (+/+) littermates of Lurcher mutants are completely healthy and are used as ideal controls.

In our laboratory we use two strains of these mice – C3H and C57Bl/7. In both there is the same type of mutation and neurodegeneration. In spite of it they differ in many parameters. There are marked strain differences in spatial learning ability [5, 6]. Mice of the C3H strain, compared with that of the C57Bl/7 strain, are almost unable to learn to find a hidden platform in the Morris water maze [7]. Interstrain differences of spatial learning tested in the Morris water maze are known also in laboratory rats [8]. In mice of the C3H strain a hereditary retinal degeneration determined by homozygous combination of rd1 gene (rd1/rd1) occurs [9]. Retinal degeneration leads to almost complete loss of photoreceptors before animals reach the age of 30 days.

The aim of this work was to assess, whether the worse result of C3H mice in spatial learning are linked to the retinal degeneration.

Materials and methods

Two types of experiments in the Morris water maze were arranged. In the first one (experiment 1) the animals learned to find a platform hidden under the water surface. Four trials a day were performed from different starting points marked as imaginary cardinal points. The position of the platform was changed from trial to trial, though it was placed always in the centre of one of the quadrants delimited by the starting points. On the wall of the maze there were mounted two labels (15 x 15 cm) with black and white vertical stripes. One of the labels was placed on the maze wall in the middle of the margin of the quadrant in which the platform was localised. The width of stripes on this label was 0.5 cm. The second label was on the margin 90° to the left or right from the first label and stripes width was 1.5 cm. The label with narrower stripes indicated the position of the platform and the mice had to differentiate between the two labels. This experiment was divided into two parts – on the first five days and the day after two days pause (D1-D5, D8) there was no change in the width of the stripes. From the next day (D9) the stripes width on the label, which did not indicate the platform, changed gradually so that in the last day of the experiment (D12) the stripes on both labels were the same. Time of reaching the platform (escape latency) in the individual days of the experiment was measured.

According to the results obtained in the Morris water maze test, some animals were taken for histological examination of retina to trace relation between retinal degeneration and spatial learning.

In the second experiment (experiment 2) the swimming velocity was measured and the strategy of maze exploration was assessed. Morris water maze without the platform was used in this experiment. A circle, which divided the maze into concentric central and peripheral zone of the same areas, was defined. The mice were trained in the maze four times a day for 60 s consecutively from individual starting points in 10-min intervals. Movements of the mice in the maze were registered by EthoVision system. Swimming velocity and time spent in the central zone was measured. Retina of all animals involved in this experiment was examined histologically.

The animals taken for the histological examination were sacrificed by Thiopental overdosing. Their eye bulbs were extracted and stored in 4% paraformaldehyde for several days for fixation. Then the eyes were sectioned with cryostat (16 μm). The sections were stained with hematoxilin-eosin. Retina was examined microscopically. Its degeneration was identified according to missing outer nuclear layer. Each mouse was then classified according to the presence or absence of the retinal degeneration.

Adult Lurcher mutant and wild type mice of the C3H strain were used (12 Lurchers and 16 wild type mice in the experiment 1, 23 Lurcher mutants and 26 wild type mice in the experiment 2). They were kept at 12:12 light: dark cycle. Food and water were accessible ad libitum. For statistical analysis of measured data Mann-Whitney test was used.

Results

The group of wild type mice showed significantly shorter latencies compared to Lurcher mutants in the experiment 1, however, some of the mutants were able to find the platform earlier than some of the wild type mice (Fig. 1). It means the distribution of latencies did not create two clearly separated groups. Latency shortening – manifestation of learning – was observed neither in mutants nor in

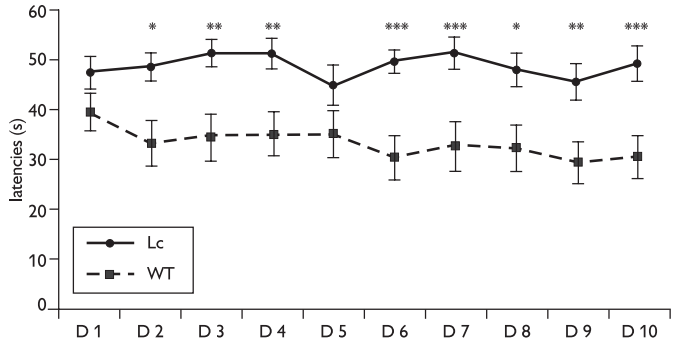

*Figure 1 – Mean latencies (s) in Lurcher mutant (Lc) and wild type (WT) mice in individual days (D1-5, D8-9) of the experiment 1. Error bars represent standard error of the mean. *p < 0.05, **p < 0.02, ***p < 0.001*

wild types. The change of the stripe width in the second part of the experiment did not have any effect on the latencies.

Mice of both types with good results in the experiment 1 had no histological evidence of retinal degeneration (Fig. 2). In animals with worse results in the maze, the histological examination confirmed degeneration of retina (outer nuclear layer was completely absent) (Fig. 3).

Swimming velocity was nearly the same in all groups of mice at the beginning of the experiment 2 (Fig 4). On the fourth day there were moderate differences. In wild type mice affected with retinal degeneration the velocity decreased a bit, while in affected Lurchers it increased as compared with unaffected animals. In healthy wild type mice time spent in the central zone was higher at the beginning of the experiment 2 and shortened on day 3 and 4. In animals with retinal degeneration the time was shorter and did not change during the course of the experiment (Fig. 5A). Contrary, in Lurchers affected with the retinal degeneration time spent in the central zone was longer than in Lurchers with normal retina (Fig. 5B).

Discussion

Retinal degeneration influences the strategy of maze exploration and causes worse results in the water maze. Because outer nuclear layer, the photoreceptors, is missing, we can suppose that mice affected with the retinal degeneration are practically blind. If the blindness is total or not we cannot decide. Papillary reaction to light is present even in mice with retinal degeneration. The reaction is mediated by several remaining photosensitive cells. Most of trials in the maze with the platform were unsuccessful. Mice with the retinal degeneration reached the platform only several times, probably randomly, while individuals with normal retina were able to find it more frequently. It indicates that they are unable to use visual information to localise the platform. Marked histologically detectable

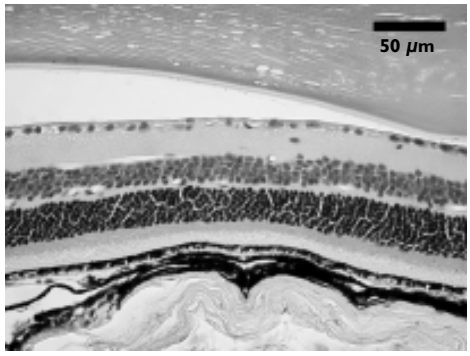

Figure 2 – Normal mouse retina, hematoxylin--eosin.

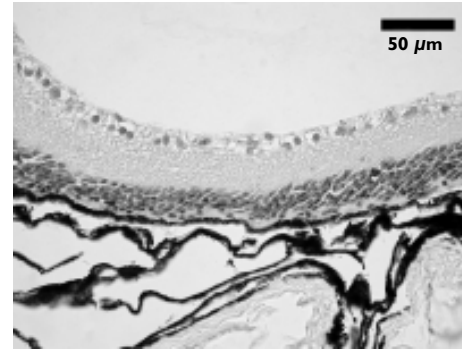

Figure 3 – Mouse retina affected with the degeneration, outer nuclear layer is absent, hematoxylin-eosin.

changes in the retina and behaviour in the maze suggest that vision affection by the retinal degeneration is quite severe, however we are not able to set it exactly.

Neither in wild type mice nor in Lurchers shortening of latencies (a sign of learning) was observed. The groups of Lurchers and wild type mice contained both animals affected and unaffected with the retinal degeneration. That is why some shortening of latencies could be expected thanks to individuals with normal retina. In Lurchers this fact can be explained by their cognitive deficit due to the olivocerebellar degeneration in agreement with the theory, that the cerebellum is involved in cognitive functions [10, 11, 12]. Wild type mice should be able to learn. In our experiment there were two labels in the maze. Only one indicated the platform position. The mice were probably not able to differentiate between them and they were confused. In the second phase of the experiment when the labels became gradually of the same appearance, the latencies were not changed. It also indicates that the width of the stripes was indifferent for the mice and that they did not differentiate the labels even if their retina and probably also their visual functions were normal.

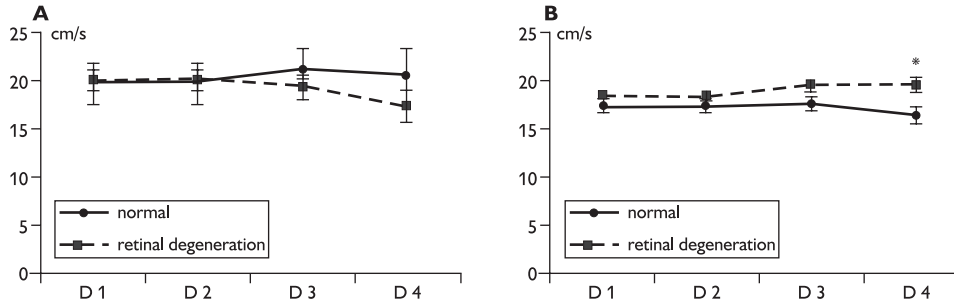

*Figure 4 – Comparison of swimming velocity (cm/s) in mice with normal retina and that with retinal degeneration in – wild type (A) and Lurcher mutant (B) mice. Error bars represent standard error of the mean. *p < 0.02*

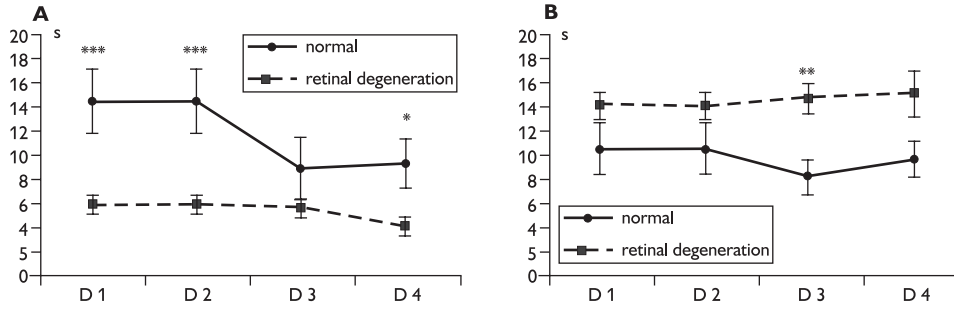

*Figure 5 – Comparison of time spent in the central zone (s) in mice with normal retina and with retinal degeneration in – wild type (A) and Lurcher mutant (B) mice. Error bars represent standard error of the mean. *p < 0.02, **p < 0.01, ***p <0.002*

The second experiment showed that when introduced for the first time into the water maze, the swimming velocity is the same in both mice with degenerated and normal retina. On the contrary the strategy of the new space exploration is different. Time spent in the central zone indicated whether the mice explored the middle of the maze or whether they preferred its periphery and tried to climb up on the wall. The effect of visual affection on the exploration strategy was completely different in wild type and Lurcher mutant mice. While in wild type mice affected individuals preferred strongly the periphery and healthy ones explored more the middle of the maze, with the retinal degeneration affected Lurchers left the margin for longer time than unaffected Lurchers.

Conclusion

The retinal degeneration influences negatively visual orientation, so that C3H strain is not suitable for experiments demanding visual orientation without considering this fact. Since the retinal degeneration is not present in all C3H strain mice, animals with intact retina can be used for such kind of experiments. Presence of the retinal degeneration can be estimated by behaviour of the animal in the water maze and proved by histological examination after finishing the behavioural experiments.

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