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Preliminary Study of the Effect of Repeated Motor Training on Spatial Learning Ability in Adult Lurcher Mutant Mice

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Abstract: Lurcher mutant mice represent a model of olivocerebellar degeneration. They suffer from cerebellar ataxia and deterioration of cognitive functions. The aim of the work was to study the effect of repetitive enforced motor training on spatial learning ability and motor coordination in adult Lurcher mutant mice of the C57Bl strain. Experimental mice were trained repetitively on a rotarod. Control mice were left without the training. Motor coordination was tested four times-before the training, in the third week of the training, at the end of the training and after a spatial learning test following the training. A rotarod of higher cylinder diameter and lower rotation speed was used. Spatial learning was examined using the Morris water maze. Trained animals achieved significantly better results than untrained mice in the 2nd and 3rd motor coordination test. In the last test following the spatial learning examination, untrained mice improved their performances so that there were no differences between trained and untrained group. In the Morris water maze trained mice showed higher spatial learning ability than untrained animals. Motor coordination capability of adult Lurcher mutant mice was improved by the training on rotarod but also by swimming during the experiment in the water maze. Repetitive motor activity led to increase of spatial learning ability.

Introduction

Lurcher mutant mice represent a natural model of olivocerebellar degeneration [1]. It is caused by a mutation in the δ2 glutamate receptor subunit gene, which is specifically expressed in cerebellar Purkinje cells [2]. Heterozygous individuals (+/Lc), the Lurcher mutants, suffer from complete postnatal loss of Purkinje cells and substantially decreased number of cerebellar granule cells and inferior olive neurons. Purkinje cells' death is a primary effect of the mutation and the cells became extinct due to excitotoxic apoptosis induced by glutamate stimulation of the abnormal receptor [3]. The death of granule cells and inferior olive neurons is secondary to the loss of Purkinje cells. The degeneration is completed at postnatal day 90, when there are no Purkinje cells in the cerebellum of Lurcher mutants and only 10 % of granule cells and 25 % of inferior olive neurons remain [4].

Lurcher mutant mice are affected by cerebellar ataxia [5, 6], deterioration of cognitive functions including spatial learning [7, 8], higher CNS excitability and changed sensitivity to painful stimuli [9, 10]. Some of their neurones are more sensitive to neurotoxic agents [11]. Wild type (+/+) littermates of Lurchers are completely healthy. Affected homozygots (Lc/Lc) are not viable due to a massive loss of neurons in the brainstem during the prenatal development [12, 13]. Lurcher mutant mice are used as a model of functional cerebellar decortication. Because of the missing Purkinje cells, these mice loose the output from the cerebellar cortex projecting mainly to cerebellar deep nuclei, though there were not found any functional problems related to these nuclei [4] and any morphological defects of them [14].

Physical activity and training are beneficial in many neurological diseases, not only those affecting motor functions, but also in cognitive deficits. There are evidences in literature sources that physical activity influences neuronal and cognitive plasticity [15] and enhances cognitive functions in both animals and humans [16, 17].

The aim of the work was to study the effect of repetitive enforced motor training on spatial learning ability and motor coordination in adult Lurcher mutant mice of the C57Bl strain.

Materials and Methods

Adult (older than 60 days) C57Bl Lurcher mutant mice of both sexes were used (males and females were distributed equally into both experimental groups). They were kept in 12/12 hour light/dark cycle. Food and water were available *ad libitum*. The mice were housed individually in plastic cages (11 × 25 cm, 14 cm high) with a metal mesh cover during the course of the experiment. 11 animals were trained repetitively on a rotarod. 16 mice were untrained controls.

In total 32 training sessions, 4 motor coordination tests and 10 days spatial learning test were performed according to the schedule shown on Figure 1.

For training a rotarod with cylinder diameter 4 cm and rotation speed 4 turns per minute was used. The training started 12 days after the first motor coordination test and it was performed for 6 weeks 5 days a week and for next 2 days in the 7th week. The mice spent on the rotarod 2 minutes four times a day. If a mouse fell down time measurement was interrupted automatically and continued again when the mouse was placed back on the rotarod. Between the four trials mice spent 6–8 min resting in their home cages.

Motor coordination was tested using a rotarod of different parameters – cylinder diameter 17 cm and rotation speed 1 turn per minute. The test was performed four times (T1–T4): 12 days before the beginning of the training, and 4, 8 and 10 weeks after the first test. In one session the trial was repeated four times in 8 min intervals. A trial was considered as successful when the animal did not fall down within 60 s or if it left the rotarod actively (it jumped down). Fall down latencies and success rate were evaluated. In case of active jump, the maximal latency (60 s)

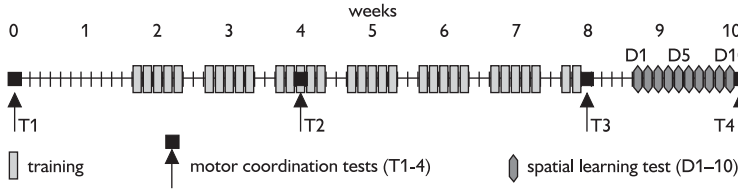

Figure 1 – Time scheme of the experiment. The experiment begins with the first motor coordination test (T1) and is finished with the fourth motor coordination test (T4) 71 days later.

was set for evaluation of mean latencies. The Mann-Whitney test was used to compare results of trained and untrained mice.

Spatial learning was examined using the Morris water maze method [18]. The test of spatial learning started 6 days after the last training day and was repeated for 10 consecutive days (D1–D10). A round pool of diameter of 95 cm was used. Water depth was 21 cm and the wall height above the water level was 9 cm. The water temperature was set at 29 ± 1°C. On the margin of the pool four starting points were marked as imaginary cardinal points. A round glass platform (7.5 cm in diameter) was placed in the middle of the south-west quadrant. The platform was submerged 0.5 cm under the water surface so that it was not visible for swimming mice and they had to localise it according to distal landmarks. Four trials a day were performed. Mice were released into the maze consecutively at all four starting points in the following order north, south, west and east. If the mouse did not reach the platform within 60 s it was placed there. Each mouse was left on the platform for 30 s. The mice spent 15 minutes resting in their home cages between the trials. Movements of the mice were recorded by the automatic tracking system EthoVision (Noldus, Netherlands). Latencies of the platform reaching, swimming distance and swimming velocity were evaluated. ANOVA for repeated measures was used to compare the development of measured parameters (learning curves) in trained and untrained mice.

All experiments were performed in full agreement with the EU Guidelines for scientific experimentation on animals and with permission of the Ethical Commission of the Faculty of Medicine in Pilsen.

Results

In the first motor coordination test done before the training, animals of both groups did not differ. Both the trained and untrained mice subsequently improved their performance. The amelioration was more marked in the trained animals and in the second and third motor coordination test they reached significantly better results as compared with untrained mice (Figure 2) – trained mice showed higher

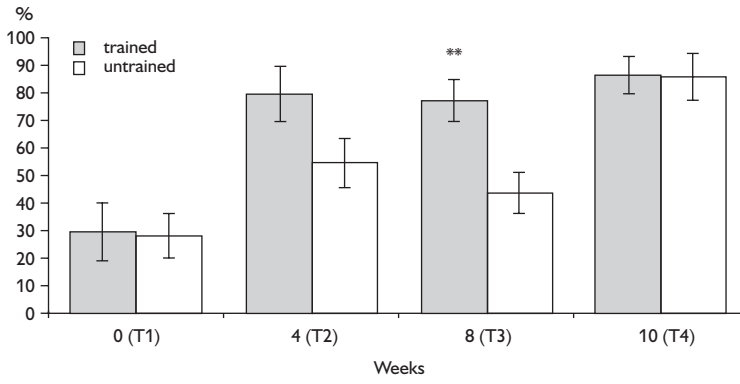

Figure 2 – Mean success rate in % ± standard error of the mean in 4 sessions (T1–T4) of the motor coordination tests in mice trained on a rotarod and untrained animals.

success rate ($U=36.0$, $p<0.02$ in the 3rd test – T3) and longer latencies ($U=45.0$, $p<0.05$ in the 2nd test – T2 and $U=44.0$, $p<0.05$ in the 3rd test – T3). In the last motor coordination test following the spatial learning examination, untrained mice strongly improved their skills so that there was no difference between the trained and untrained group.

In the Morris water maze the trained mice were more successful as compared with untrained ones. Their latencies and swimming distance were significantly shorter ($F=25.49$, $p<0.00004$ and $F=27.53$, $p<0.00002$ respectively) than those in untrained controls (Figure 3 A, B). Swimming velocity was slightly lower in trained mice than in untrained animals ($F=6.18$, $p<0.02$) (Figure 3 C).

No differences were observed between the males and females in both motor coordination tests and Morris water maze (data not shown).

Discussion

The first motor coordination test showed that both groups of mice had the same abilities when uninfluenced by the training. As the groups were created by random selection of animals this fact was expected and it confirms that initial conditions were equal for both groups of mice. Slight improvement of motor coordination ability in the following two sessions in untrained mice could be attributed to motor learning. In the second group of mice training on the rotarod led to more marked increase of motor skills in comparison with untrained animals. Although the rotarod used for the training was different than that one used for the motor coordination tests, the motor patterns in both of them were similar and motor learning could take part in the improvement in trained mice too. It is known that Lurcher mutants have motor learning ability in spite of the cerebellar degeneration [5].

The last motor coordination test was done after the spatial learning examination. The experiments in the water maze were performed for 10 days and they were repeated four times a day. In each trial the mice swam for several seconds at least (on the first days nearly 60 s) until they reached the platform. In fact, the experiment in the water maze represented enforced swimming. It substituted completely the effect of the training on the rotarod in untrained mice and they reached the same motor abilities as trained individuals, despite the time of enforced swimming was much shorter than time spent with rotarod training and the motor patterns of swimming were completely different from movements on the rotarod.

In trained mice no significant changes were observed between the second, third and fourth motor coordination test. It the second test they reached their best results. It shows that the first 12 training days were sufficient to improve motor skills of Lurcher mice to high level so that next improvement was not possible to achieve either by prolonged rotarod training or by enforced swimming.

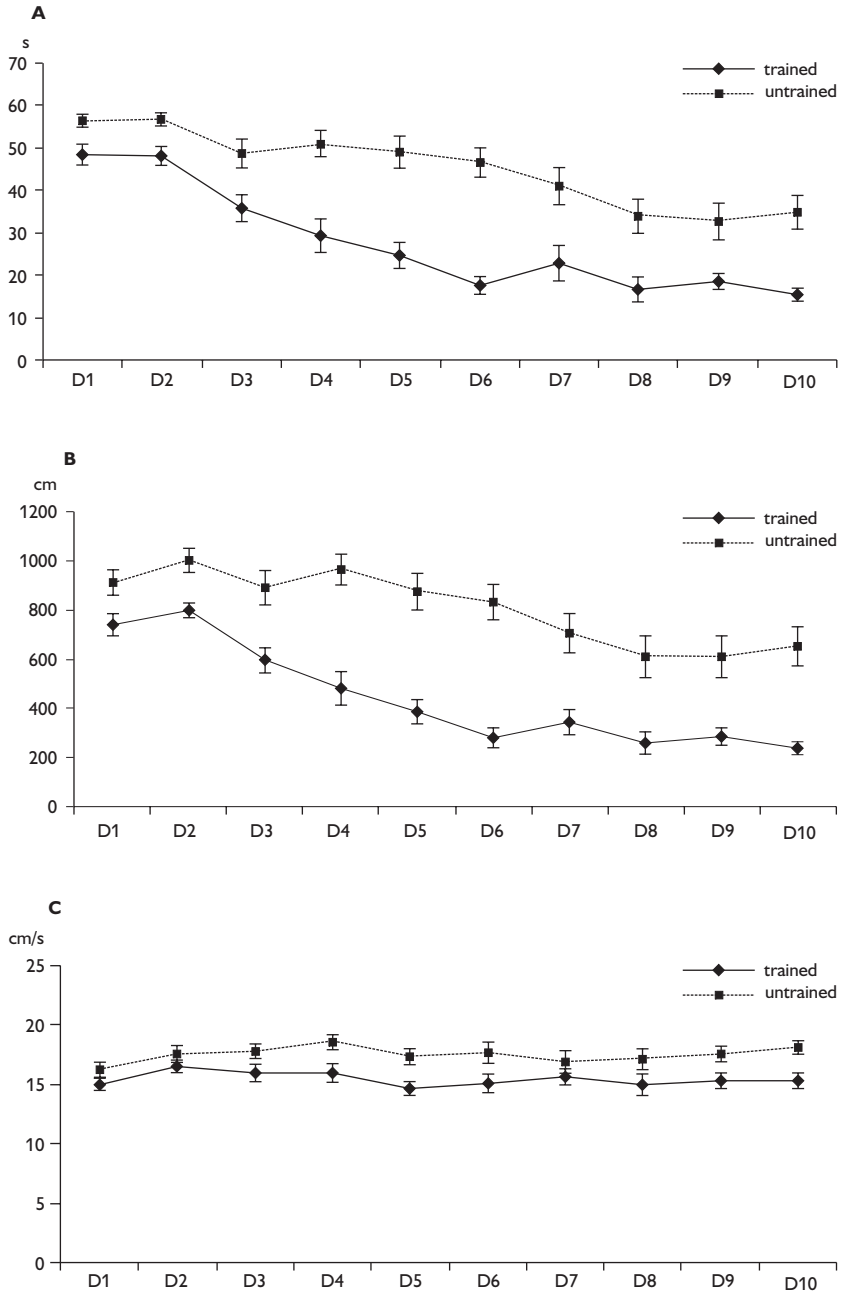

Figure 3 – Mean latencies (in s) ± standard error of the mean (A), mean swimming distance (in cm) ± standard error of the mean (B) and mean swimming velocity (in cm/s) ± standard error of the mean (C) in the 10 days (D1–D10) experiment in the Morris water maze in mice trained on a rotarod and untrained animals.

Long-term enforced motor activity led to significant increase of spatial learning ability in adult Lurcher mutant mice. Manipulation with the mice, transport in the laboratory and contact with researchers during the training provided environmental enrichment and worked as a handling at the same time. Both these factors, environmental enrichment and handling, could influence the cognitive functions in laboratory animals too. Environmental enrichment is known to enhance cognitive functions [19] and mitigate deficits in cognitive functions in some mutant animals with CNS defects, e.g. in a mouse models of Alzheimer's disease [20] or Huntington's disease [21]. It can even enhance neurogenesis in hippocampus in mice [22].

While swimming velocity in trained mice was lower than in untrained ones, shorter latencies found in trained mice were not due to their better physical capacities and they should be attributed to higher learning ability. This fact is also supported by shorter (more direct) swimming trajectory between the starting point and the goal (platform) in the water maze.

Conclusion

Enforced motor activity and training improved motor abilities in adult C57Bl Lurcher mutant mice suffering from cerebellar ataxia. Short training or enforced activity with different motor patterns was sufficient for maximal effect in Lurchers and next prolongation was ineffective. Long-term and repeated physical activity, handling or enriched environment increased significantly spatial learning ability in adult Lurcher mutant mice. Enforced swimming sufficiently substituted much longer enforced motor rotarod based training.

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