Measuring Equilibrium and Motor Learning Deficits during Aging with the Elevated Unsteady Board in Mice with Precocious Cerebellar Degeneration

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Abstract

Lurcher mutant mice are well known to exhibit massive and neonatal degeneration of Purkinje cells in the cerebellar cortex which influences normal ageing process. In spite of their severe motor disturbances, at 3 months old, these mice are still able to learn a motor task. We evaluated such abilities during aging with the Elevated Unsteady Board test. The results clearly showed that the precocious and focused degeneration of Purkinje cells have mild and long term consequences on motor learning functions in these mutants.

Introduction

Many tests exist to investigate motor abilities in rodents. Some of them aim at measuring motor coordination and equilibrium abilities when the animals are free to move (i.e. the hole board and the parallel rod floor tests). On the contrary on other tests animals are forced to adjust their posture in response to a moving surface (i.e. the rotorod test). Nevertheless, in these entire tests animals can move to maintain balance and equilibrium evaluation is made on dynamic conditions. The Elevated Unsteady Board was created in our lab to measure equilibrium maintenance in response to a moving surface but on static condition (Hilber, Lalonde, & Caston, 1999): only movement of the animal could provoke equilibrium disturbance and only adapted and quick postural body adjustment permit to avoid the equilibrium loss and fall. This test permitted to show that the cerebellar Lurcher mutant mice exhibited equilibrium disturbances on static conditions. These mutants are well known to exhibit complete degeneration of Purkinje cells in the cerebellar cortex (Vogel, Caston, Yuzaki, & Mariani, 2007) which begins very early in development stages and influences normal ageing process (Hilber & Caston, 2001). Here we used the Elevated Unsteady Board apparatus to investigate the effect of daily training on equilibrium abilities in the cerebellar Lurcher mutant mice during aging.

Material and protocol

Animals

The mice were obtained in our laboratory and reared in standard conditions (12h light (8am-8pm)- 12h dark (8pm-8am), 21°C, food and water available *ad lib*. Heterozygous Lurcher (+/Lc) were obtained crossing females of wild type of B6CBA strain (+/+) with heterozygous Lurcher males of the same strain. 80 animals were tested, split in four groups of ages (3, 9, 15 and 21 months), containing 10 +/+ and 10 +/Lc.

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Description of the elevated unsteady board

As shown in the photo near to the Figure 1, the unstable board was circular (diameter, 8.5 cm), made of aluminium, and set at a height of 1 m from the floor. A foam rub mat was disposed on the floor to cushion animal's falls. The surface of the platform was covered with a plastic sheet for the purpose of preventing excessive sliding. The platform was positioned on a ballpoint, causing a tilt of 30° in any direction whenever the mice strayed from the middle part. The minimal weight necessary to incline the platform downward was 4g.

Protocol

At the beginning of the test, each mouse was placed in the middle of the platform, which was in a horizontal position. Thus, only motions of the animal could provoke tilting of the platform and only adapted repartition of muscular strength in the limbs and the body could permit the mouse to restore equilibrium and to maintain balance. The trial ended when two or more paws of the mouse were out of the circumference of the platform or when the mouse reached the cut off period (180 sec.). Each mouse was subjected to 3 trials per day. The intertrial interval was 15 min. The mice were trained daily until they reached the learning criterion fixed at three consecutive trials of 3 min. If they didn't reach this criterion after 10 days, the training was stopped. During each trial, we measured the time until falling. For each animal, the scores of the 3 daily trials were averaged to get the mean score per day. Then, we calculated the mean daily score (+/- sem) obtained by each group of mice. The mean scores were given +/- SEM ($\sigma/\sqrt{n}$).

Statistical analysis

Three way ANOVA and appropriate post hoc comparisons with Tukey HSD (High Significant Difference) test were used for the purpose of statistical analysis. In all instances $p<.05$ was considered as significant.

Ethical statement

All the experiments reported here were conducted in accordance with the ethical recommendations of the Directive 2010/63/EU of the European Parliament and of the Council for the protection of animals used for scientific purposes.

Results

2x4x10 ANOVA (2 genotypes, 4 groups of age, 10 days, with repeated measures on the last factor) revealed a significant training [$F(9,648) = 34.2$; $p<0.001$], genotype [$F(1,72) = 565.23$; $p<0.001$] and an age effects [$F(3, 72) = 10.1$; $p<0.001$] with significant age x genotype x repeated measures interaction [$F(27, 648) = 7.3$; $p<0.001$], which indicates that the evolution of the performances during training differed for the two genotypes as a function of age (see Figure 1). Whatever the age, the scores performed by the mutants during the first day that is before training were lower than those of controls ($p<0.001$). Whereas the scores of +/+ mice significantly decreased between 3 and 15 months ($p<0.001$), no significant decrease of performances was observed in Lurcher mice with age: concerning the scores performed during the last day (deficits after training), the scores of +/Lc mice were always lower than those of controls whatever the age ($p<0.05$ at 3 months and $p<0.001$ at 9, 15 and 21 months). The performances did not decrease with age in +/+ mice (they were maximal at all ages) but decreased significantly in the +/Lc mice as soon as 9 months (3 versus 9 months: $p<0.001$).

Discussion

The Lurcher mutants are an interesting tool to observe the consequences of a focused neurodegeneration on ageing processes (Hilber & Caston, 2001). The results of this study showed that in spite of their equilibrium

disturbances on static conditions the young mutants were still able to elaborate and execute a novel equilibrium motor task on static conditions. Nevertheless although the latency before falling from the platform improved with training, the scores of the mutants were always poorer to those of controls, indicating motor learning deficits in the mutants. Equilibrium abilities evaluated before training decreased in +/+ mice indicating age-related impairments. Such impairments were not observed in +/Lc mice certainly because of a floor effect. In spite of their equilibrium deficits, all the +/+ mice reached the learning criterion, indicating that if their motor abilities were affected by the age, their motor learning abilities were not: they were still able to elaborate and to execute a motor program which permitted them to stay immobile on the unstable platform to avoid the fall. The results are on the opposite in the Lurcher mice since, as mentioned above, their equilibrium abilities were not significantly deteriorated but their motor learning decreased as soon as 9 months old. Thus, with age, these mice became progressively unable to elaborate and execute a novel motor program adapted to the task: on the rotorod the walking strategy disappeared (Hilber & Caston, 2001) and, on the unstable platform, they became unable to use muscular synergies which would permit to stay immobile on the apparatus.

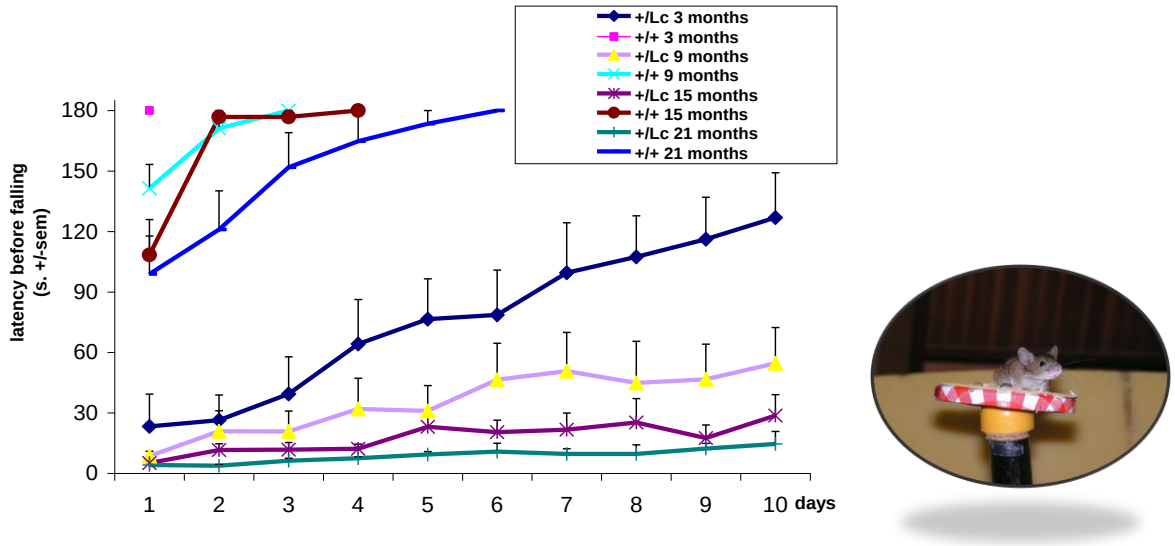

Figure 1. Evolution of the performances (latency before falling) of +/+ and +/Lc mice on the Elevated Unsteady Board during training (days).

References

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