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CHANGES OF MOTOR ABILITIES DURING ONTOGENETIC DEVELOPMENT IN LURCHER MUTANT MICE

V. MARKVARTOVÁ,* J. CENDELÍN AND F. VOŽEH

Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University in Prague, Lidická 1, 301 66 Plzeň, Czech Republic

Abstract—Lurcher mutant mice represent a natural model of olivocerebellar degeneration. This degeneration is caused by a mutation of the gene for the δ2 glutamate receptor. Lurcher mutants suffer from cerebellar ataxia and cognitive functions deficiency as a consequence of excitotoxic apoptosis of Purkinje cells in the cerebellar cortex and a secondary decrease of granule cells and inferior olive neurons. This process finishes by the 90th day of postnatal life, but already by 14 days, the Purkinje cells are damaged and the ataxia is fully developed. Purkinje cells die by apoptosis within the first 3 weeks of life. The aim of our work was to study the development of motor functions in the course of the ontogenetic development in Lurcher mutant mice of the B6CBA strain and to compare it with wild type mice of the same strain. Mice aged 2, 3, 6, 9, and 22 weeks were used in our experiment. Motor skills were examined using four standard tests: the horizontal wire, rotating cylinder, footbridge and slanting ladder. Our findings in Lurcher mutant mice show a significant increase of motor abilities up to the sixth postnatal week and selective decrease early after this period. This improvement of motor skills is caused by the physiological development of musculature and the nervous system, probably with some contribution of plasticity of the maturing brain. The cause of the decline of these abilities immediately after the completion of the development is unknown.

Key words: cerebellar ataxia, motor skills, olivocerebellar degeneration.

Lurcher mutant mice represent a natural model of genetically-determined olivocerebellar degeneration (Phillips, 1960). They serve as an exquisite model of functional cerebellar decortication. For that reason, they are used for the investigation of cerebellar functions, functional and morphological symptoms of the cerebellar degeneration and the methods of therapeutic intervention in the neurodegenerative process and its consequences. The degeneration is caused by a mutation of the gene for the δ2 glutamate receptor subunit (Zuo et al., 1997). This receptor subunit type is expressed predominantly by cerebellar Purkinje cells (Araki et al., 1993).

Lurcher mutant mice (Lc) are in fact heterozygous individuals (+/Lc), obtained by the crossbreeding of Lurcher and wild type mice. In Lurcher mutants the stimulation of the abnormal receptor by glutamate induces the excitotoxic apoptosis of Purkinje cells in the cerebellar cortex (Zuo et al., 1997). A secondary consequence of the Purkinje cells' death is the decrease in number of granule cells and inferior olive neurons because of the lost target of their axons (Wetts and Herrup, 1982a,b; Caddy and Biscoe, 1975, 1976; Heckroth and Eisenmann, 1991). A decrease in the external granular layer is seen as early as 2 days in the lobulus simplex and by 6 days of age in the uvula. Granule cell death appears than from 2 to 18 days of age (P2–18). Loss of granule cells is reflected in reduced growth of the molecular and granular layers. Purkinje cell abnormalities appear at three to four days after birth (P3–4) in the form of crowding failure of nuclear growth, and condensed or lessened cytoplasm; Purkinje cell death is apparent at four to six days of age (P4–6) depending on the region of the cerebellum followed by the death of olive neurons (Swisher and Wilson, 1977). After P10, the rate of parallel fiber synaptogenesis declines when compared to wild type mice (Dumesnil-Bousez and Sotelo, 1992; Heckroth et al., 1990). Purkinje cell number reaches 10% of normal at 26 days. At P60 there are almost no Purkinje cells in the cerebellum and the degenerative process is finished within P90, when the loss of Purkinje cells is nearly complete and only 10% of the granule cells and 30% of the inferior olive neurons remain (Caddy and Biscoe, 1976, 1979). The Purkinje cells axons are the only efferent pathway of the cerebellar cortex, so that the loss of these cells leads to complete functional cerebellar decortication. Therefore Lurcher mutants suffer from the cerebellar ataxia (Lalonde et al., 1992), spatial learning and orientation deficiency (Lalonde et al., 1988; Cendelín and Vožeh, 2001; Cendelín et al., 2008) and an altered execution of their conditioned eyelid reflexes (Porras-Garcia et al., 2005). The ataxia is manifested at the end of the second postnatal week. Changes of hippocampal long-term potentiation were found in Lurcher mutants (Barcal et al., 2001, 2002), though their hippocampus is macroscopically intact (Cheng and Heintz, 1997). They also show a higher CNS excitability (Cendelín and Vožeh, 1999; Vožeh et al., 2002) compared to the wild type mice.

Homozygous individuals (Lc/Lc) are not viable and die shortly after birth because of a massive loss of midbrain and hindbrain neurons during intrauterine development (Cheng and Heintz, 1997; Resibois et al., 1997). Wild type (WT) homozygotes (+/+)-healthy littermates of Lurcher mutants are used as ideal controls because they have the same genetic background.

*Corresponding author. Tel: +420-377-593-365; fax: +420-377-593-369.
E-mail address: vera.markvartova@lfp.cuni.cz (V. Markvartová).
Abbreviations: Lc, Lurcher; *p*, level of statistical significance; P, postnatal day; SEM, standard error of the mean; w (w2–w22), weeks (2–22 weeks old); WT, wild type.

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The aim of our work was to compare the evolution of motor functions in the course of the ontogenetic development in Lurcher mutant and wild type mice of the B6CBA strain.

EXPERIMENTAL PROCEDURES

Animals

Both Lurcher mutants and wild type mice derived from the B6CBA strain were obtained by crossing +/+ females and +/Lc males.

The mice were housed under standard conditions of temperature (23±1 °C), humidity (40–50%) and lighting (lights on 6:00–18:00 h daily) in plastic cages (30 cm×25 cm×14 cm high) with a metal mesh cover in number 2–4. Water and food were available *ad libitum*. Both Lurcher mutant and wild type mice divided into five different age groups: 2-weeks (w2, 10 Lc, 10 WT), 3-weeks (w3, 10 Lc, 10 WT), 6-weeks (w6, 10 Lc, 10 WT), 9-weeks (w9, 9 Lc, 10 WT), and 22-weeks (w22, 10 Lc, 10 WT) were tested in the experiment. Animals of both sexes were used equally, except w6 in Lc (five male, four female).

Animals of both types (Lc, WT) were subjected to an examination of motor skills by four standard tests: wire suspension, rotating cylinder, footbridge, slanting ladder. Fall latency was measured so that the criterion of success (in staying there) was 60 s. Active leaving a tool (possible practically only on the slanting ladder) was assessed as the latency of 60 s. The strategy of the animals to maintain on the tools was also observed. In the relevant age (2, 3, 6, 9 or 22 weeks), the mice were confronted with the tasks without previous experience, always only one time. Each of the animals was tested successively on all four tasks in sequence as described above. The tests were performed at the same time in the morning.

Tools

Wire suspension. This test enables the evaluation of the muscular strength of the animal by hanging it in the middle of a thin string (diameter 1 mm, length 43 cm, 40 cm above a cushioned table) by their forepaws.

Rotating cylinder. On this device the equilibrium ability and motor coordination of the animal on a wide slowly rotating cylinder (diameter 17 cm, length 20 cm, 1 rpm) were evaluated after positioning it there with its head in the direction of the rotation.

Footbridge. On this device the equilibrium ability and motor coordination of the animal were tested on a narrow footbridge (height 40 cm above a cushioned table, length 30 cm, width 2 cm) after placing it in the middle of it with its body axis perpendicular to the beam long axis.

Slanting ladder. This method permits the evaluation of the same capabilities, not only on a narrow tool, but also slanting. With its head up, the mouse was placed in the middle of the ladder (30 cm long, 4 cm wide, inclination of 50°, distance between stairs 1 cm).

Statistics

Because the data did not show normal distribution (verified with the Kolmogorov–Smirnov test), non-parametric tests were used for the statistical analysis. The dependence of the latencies on experimental groups was evaluated with the Kruskal–Wallis test (non-parametric ANOVA). The differences between the individual groups of animals were then assessed using the Man–Whitney test as a post hoc test.

All 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.

RESULTS

Fall latencies in all motor tests depended significantly on the experimental groups (Lc, WT, age groups) [Kruskal–Wallis test: $P<0.0001$].

Lurcher mutant mice achieved significantly worse results than wild type mice in all tests at least in some age periods (Fig. 1A–D, Table 1). The most marked differences were on the footbridge and slanting ladder (Table 1).

Wild type mice

On the horizontal wire, wild type mice did not come near the criterion before w6 and their performance in w2 and w3 was significantly lower then in w6 [w2/w6 $P<0.004$, w3/w6 $P<0.009$] (Fig. 1A). Also on the rotating cylinder, the performances of the animals approximated to the criterion in w6, but the initial deficit was smaller than on the wire and therefore there were no significant differences w2 versus w6 and w3 versus w6 (Fig. 1B). On the footbridge and slanting ladder, the animals had already reached the criterion at the lowest age (w2) (Fig. 1C, D). From w6 they maintained their skills on the same level during the whole observed life period (Fig. 1A–D).

Lurcher mutant mice

Lurchers were practically unable to stay on any device more than 10 s in average at the age of w2. Till w3 their results improved significantly on all tools (Fig. 1A–D, Table 2). While on the cylinder and footbridge, Lc had already reached the best results in w3, in the ladder and wire tests, the longest latencies were observed in w6. However, the difference between w3 and w6 was statistically insignificant in all motor tests (Fig. 1A–D, Table 2). In the wire and cylinder tests, no significant changes were found after w6. In the footbridge and ladder tests, the significant worsening of the performance was observed from w6 to w22 (Fig. 1A–D, Table 2).

DISCUSSION

Our work documents the state of motor abilities of B6CBA Lurcher mutant and wild type mice during their development from beginning of the walking activity (2 weeks) to 5 months of age.

On the horizontal wire, the performance increases from w2 to w3 respectively to w6 in both Lurchers and wild type mice and did not change significantly in the later period. The initial progress probably reflects the development of musculature and motor abilities in young animals. Though the development in Lurchers resembles the wild type mice, their motor abilities remain on a lower level than in control mice of the same age which confirms the findings of Hilber and Caston (2001). However, in contrast to their findings, we detected no significant decrease of muscle strength, neither in Lurchers nor in wild type mice in the observed age period.