Changes of Dopamine Receptors in Mice with Olivocerebellar Degeneration

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Abstract: Lurcher mutants are mice with functional mutation in the δ2 glutamate receptor (GluRδ2) that is predominantly expressed in cerebellar Purkinje cells and plays a crucial role in cerebellar functions. These mice display ataxia and impaired motor-related learning tasks. In order to elucidate the role of dopaminergic receptor system in coping with mutation in δ2 glutamate receptor the behavioral effect (spatial learning) of D_1 dopamine receptor activation and inhibition and changes in D_1-like and D_2-like dopamine receptors in striatum, hippocampus and cerebellum in C57Bl/7 and C3H Lurcher mutants and wild type mice were studied. We have found that Lurcher mutants were worse in the spatial learning but mice of both types reacted similarly to D_1 dopamine receptor agonist (without effect) and antagonist (worsening). Moreover, Lurchers revealed substantial higher density of both D_1-like and D_2-like dopamine receptors in hippocampus in C57Bl/7 strain, while in C3H strain only D_1-like dopamine receptors were higher. In C57Bl/7 strain, D-like dopamine receptors were lower in cerebellum; D_2-like dopamine receptors were not affected. In the striatum, the receptor densities were similar to the wild type counterparts. Our results suggest specific participation of dopamine receptor system in coping with olivocerebellar degeneration.

Introduction

The δ2 glutamate receptor (GluRδ2) is predominantly expressed at distal dendrites of Purkinje cells where parallel fibers form synapses [1]. It is important to note that GluRδ2 does not form functional glutamate-gated ion channels when expressed, either alone or with other glutamate receptors. Despite that functional exceptionality, GluRδ2 is crucial in cerebellar function: mice that lack the gene that encodes GluRδ2 display ataxia and impaired motor-related tasks such as eye-blink conditional learning and adaptation of the vestibulo-ocular reflex. Mice with functional mutation in the δ2 glutamate receptor gene (i.e. Lurcher mice) display cerebellar ataxia, lower resistance of the CNS against a neurotoxin [2], and impaired cognitive function [3, 4]. Despite its importance, the mechanism by which GluRδ2 participates in cerebellar functions is still unexplained.

Lurcher mutant mice are heterozygote animals (+/Lc) which suffer from progressive and complete loss of cerebellar Purkinje and granule cells, and inferior olive neurons. By postnatal day 26 there is only 10 % of Purkinje cells remaining, by postnatal day 90, there are no Purkinje cells. On that day, granule cells represent 10 % and inferior olive neurons are reduced to 25 % of figures in wild type mice [5]. Purkinje cells die in consequence of a functional mutation in the δ2 glutamate receptor gene and this is a type of excitotoxic apoptosis [6]. Affected homozygotes (Lc/Lc) are unable to survive, unaffected homozygotes – wild type mice (+/+) are healthy and serve as controls.

Dopaminergic system is one of the most important transmitter systems. It is, between other functions, tightly connected with cognitive function. Dopamine

(DA) is endogenous catecholamine acting first of all in the central nervous system. In the brain, DA acts not only as a pure neurotransmitter but also as a neuromodulator which is released both from axon terminals and dendrites (e.g. in the substantia nigra pars reticulata). The brain dopaminergic system is involved in the control of locomotion, learning, working memory, cognition and emotion but also in various neurological and psychiatric disorders such as Parkinson's disease, schizophrenia, and amphetamine and cocaine addiction [7, 8]. Furthermore, DA plays an important role in long-term potentiation (LTP) of hippocampal – prefrontal synapses and their plasticity. It participates in a remarkable and long-lasting inhibition of LTP which represents the impact of stress on cognitive functions [9]. The activation of dopaminergic system has also impact on immune functions when stimulation of DA receptors influences intensity of the immune responses in mice [10, 11, 12]. According to their structural similarities DA receptors presented by neurons are divided into two groups: D_1-like (D_1 and D_5 subtypes) and D_2-like (D_2, D_3 and D_4). All these subfamilies belong to the largest group of receptors – to G protein coupled receptor family. While D_1-like activate via Gs protein adenylyl cyclase, D_2-like family (mainly pre-synaptic) inhibit adenylyl cyclase via Gi protein activation. Moreover, coupling with Gq protein allow D_2-like group of receptor to activate phospholipase C. In this view, DA receptors represent effective system that is able to affect the target cell function when D_1-like receptor family is activated. In addition to this fact, fine tuning of the signal is possible via pre-synaptic modulation by D_2-like receptor family.

Therefore, the role of dopamine transmitter – receptor system was investigated in mice with olivocerebellar degeneration. First, the behavioral effects of D_1 dopamine receptors-activation and inhibition on spatial learning in Lurcher mutant and wild type mice derived from C57Bl/7 strain were followed. Second, the density of D_1-like and D_2-like DA receptor in three brain structures (hippocampus, striatum, cerebellum) of these animals was investigated. Third, some strain differences between C57Bl/7 and C3H mice were studied.

Material and Methods

Animals

Young adult control (WT) and Lurcher mutant mice of both sexes (+/Lc) mice (20–30 g; age 164.74±3,97 days) of two strains (C57Bl/7 – 65 experimental animals and 37 controls; C3H – 48 experimental animals and 39 controls) were used. Mice were grown in our facilities with 12/12 day cycle, food and drinking water *ad libitum*. Mice were sacrificed by decapitation and exsanguination and the brain and cerebellum were dissected, put on the frozen desk and hippocampus and striatum were dissected. Than the tissue was flash frozen in liquid nitrogen and stored at –80 °C for further analysis.

D_1 dopamine receptor agonist/antagonist application
D_1 dopamine receptor antagonist SCH 23390 was applied intraperitoneally in dose 0.5 mg/100 g of body weight (20 min. before testing of spatial learning).
D_1 dopamine receptor agonist SKF 38393 was applied intraperitoneally in the same dose 60 min before testing of spatial learning. Controls received the same volume at the same interval of physiological saline.

Spatial learning
Spatial learning was tested in the Morris water maze [13]. The apparatus was a circular pool (diameter 95 cm) filled with water. We assigned four imaginary cardinal points on the periphery of the pool. In the middle of the south-west quadrant we put a round transparent glass platform (diameter of 7.5 cm). The platform was hidden 0.5 cm bellow the water surface. Experimental animals were placed into the pool consecutively from all four cardinal points. Their task was to find the platform and to climb it up. If the mouse did not reach the platform within 60 s, we put it there. The mouse stayed on the platform for 30 s. The experiments were performed in the same manner for 6 consecutive days (D1–D6) four times daily. Latencies of reaching the platform were evaluated.

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

Binding experiments
Binding experiments were performed similarly as described previously [14]. Briefly, in preliminary experiments, the receptors were bound with increasing concentrations of radioligand in order to ascertain:

- the saturating concentration of radioligand, and
- the receptor affinity to radioligand, expressed as dissociation constant (K_D).

The radioligands used were ^{3}H-SCH 23990 (specific for D_1-like dopamine receptors; concentrations ranked from 6 pmol/l to 4 nmol/l), and ^{3}H-spiperon (specific for D_2-like dopamine receptors; concentrations ranked from 3 pmol/l to 2 nmol/l). The non-specific binding was determined using cis-flupentixol (50 μmol/l, specific antagonist of D_1-like dopamine receptors) and sulpiride (20 μmol/l , specific antagonist of D_2-like dopamine receptors), respectively. The incubations were performed in duplicates in Tris-EDTA buffer (Tris-HCl 50 mmol/l, EDTA 2 mmol/l, pH adjusted to 7.4), with final volume 500 μl and lasted 90 min at 25 °C for D_1-like dopamine receptors and 90 min at 25 °C for D_2-like dopamine receptors. The reaction mixtures were filtered through Whatman GF/B glass fiber filters and the unbound radioligand was washed out using three times wash with 3 ml of ice-cold distilled water. The filters were then placed in scintillation vials, desiccated overnight, then covered with scintillation

cocktail (Bray's solution) and stored in the dark for 2 hours to minimize chemiluminescence, before being counted.

Than, simplified saturation binding experiments with one saturating concentration of radioligand were used in order to determine the receptor density (B_{max}). The incubation procedure was the same as stated before. In order to ascertain the receptor density, following formula was used: $B_{max} = B \times ([L] + KL) / [L]$ (1), where B= bound of radioligand [fmol/mg of protein], L = radioligand concentration [fmol/l], and KL = K_D [fmol/l] of the radioligand. Homogenates were incubated in duplicates with single fully saturating concentration of ^{3}H-SCH23390 (3000 pmol/l) and ^{3}H-spiperon (2000 pmol/l), respectively.

In all cases, the protein concentration was determined according to modified Lowry's method.

Material

^{3}H-SCH23390, [N-methyl-^{3}H] (specific activity=3.15 TBq/mmol), and ^{3}H-spiperon (8-[4-(p-fluorophenyl)-4-oxo[2,3(n)-butyl]-1-phenyl 1,3,8-triazospiro[4,5]decan-4-one) [benzene ring-^{3}H] (specific activity=0.56TBq/mmol) were purchased from Perkin-Elmer, Boston, MA, USA. Other chemicals were purchased from Sigma Czech Republic.

Data treatment

Statistical analysis of reaching platform latencies in individual days (in seconds) in experimental animals compared with controls was done using ANOVA for repeated measures. Radioligand binding data were evaluated using GraphPad software. Statistical significance of differences between means was evaluated with Student t-test.

Results

Spatial learning

Lurcher mutants revealed worse results in the Morris water maze than wild type mice, when treated with physiological saline. In mice of the C57Bl/7 strain the difference was more marked ($p<4\times10^{-6}$) then in the C3H strain ($p<0.03$). With physiological saline treated C57Bl/7 wild type mice showed significantly shorter latencies as compared with C3H wild type individuals ($p<9\times10^{-5}$). D_1 receptor agonist SKF 38393 caused no changes in latencies of reaching criterion in comparison to animals treated with saline in both wild types and Lurcher mice of both strains. On the other hand the wild type animals of both strains and Lurchers of the C57Bl/7 strain that were given the D_1 receptor antagonist SCH 23390 showed highly significantly worse results in the water maze in comparison to control mice treated with physiological saline (C57Bl/7 wild type: $p<8\times10^{-11}$, C3H wild type: $p<2\times10^{-5}$, C57Bl/7 Lurchers: $p<3\times10^{-5}$) (Fig. 1A, B, C). In C3H Lurchers significant effect of SCH 23390 was not observed (Fig. 1 D).

Binding experiments

The preliminary saturation binding experiment revealed K_D to be 1223±310 pmol/l in ^{3}H-SCH23390 binding (i.e. for D1-like dopamine receptors) and 395±27.9 pmol/l in ^{3}H-spiperon binding (i.e. for D2-like dopamine receptors), respectively.

The simplified saturation experiments have shown that the D_1-like and D_2-like DA receptor densities were higher in the hippocampus of Lurcher mutants in comparison to wild type C57Bl/7 mice (Fig. 2A, B). In cerebellum, Lurcher mutants showed significantly lower density of D_1-like receptors but no differences in the density of D_2-like receptors in comparison with C57Bl/7 wild type mice (Fig. 3A, B). Finally, in the striatum no significant differences in the density of both D_1-like and D_2-like DA receptors between Lurcher mutants and C57Bl/7 wild type mice were found (Fig. 4A, B).

The situation in C3H strain differed from that of C57Bl/7 strain (Fig. 5). In the hippocampus, the density of D_1-like dopamine receptors was higher, the density of D_2-like DA receptors was not changed. On the other hand, there was no change in receptor density in the striatum. Moreover, the receptor densities differed in wild type animals of C57Bl/7 and C3H strain.

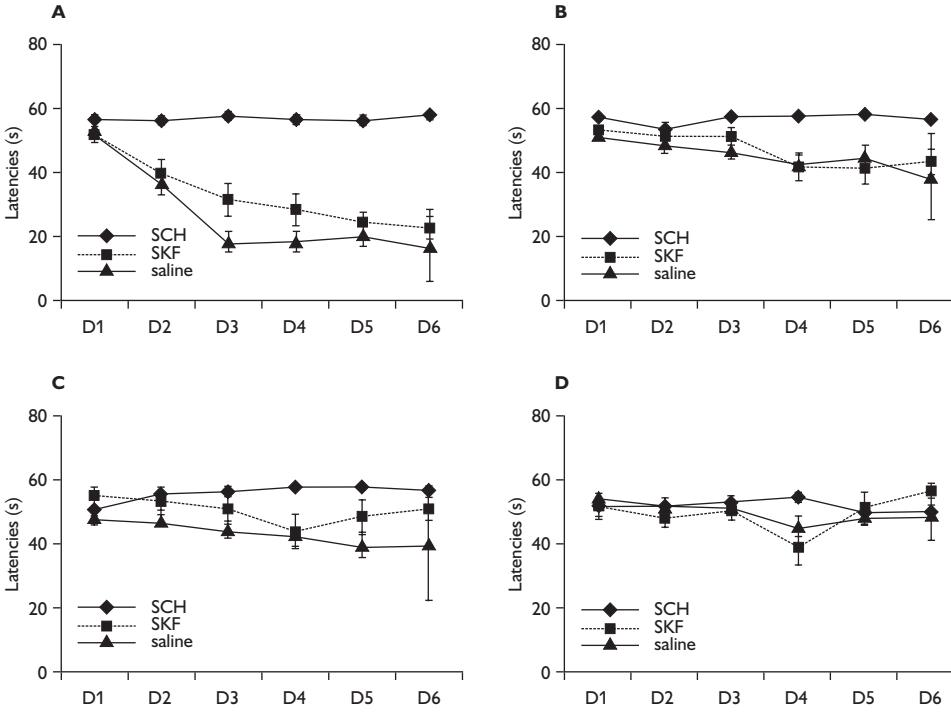

Figure 1 – Mean latencies of reaching platform in the Morris water maze in seconds ± S.E.M.(ordinate) in individual experimental days (D1–D6) (abscissa), SCH, SKF 5.0 mg/kg, physiological saline. A: C57Bl/7 wild-type mice; B: C57Bl/7 Lurcher mutants; C: C3H wild type mice; D: C3H Lurcher mutants.

Discussion

Our results have shown that dopamine transmitter – receptor system is affected in Lurcher mice. It is important to note that application of dopamine receptor antagonist SCH 23390 had similar effect in wild type and Lurcher mice. This finding, together with increased number of D_1-like and D_2-like dopamine receptors, give evidence about the role of dopamine receptors in coping with olivocerebellar degeneration. Although the Lurchers are worse in spatial learning, the function of dopamine receptor system is preserved as the changes reaching criteria were similar in Lurchers and wild types. When there would be the defect in dopamine receptor (i.e. D_1-like post-synaptic and D_2-like pres-synaptic dopamine receptors), the spatial learning should be affected in different ways in Lurchers in comparison with wild type mice. Our results are in good agreement

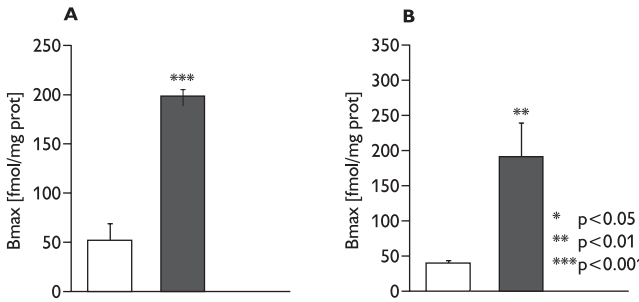

Figure 2 – DA receptor density in the hippocampus of Lurcher mutants and wild type mice. A: D_1-like receptor; B: D_2-like receptor. Wild type □; Lurcher ■

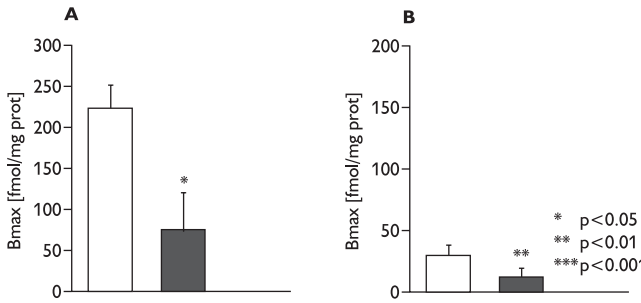

Figure 3 – DA receptor density in the cerebellum of Lurcher mutants and wild type mice. A: D_1-like receptor; B: D_2 -like receptor. Wild type □; Lurcher ■

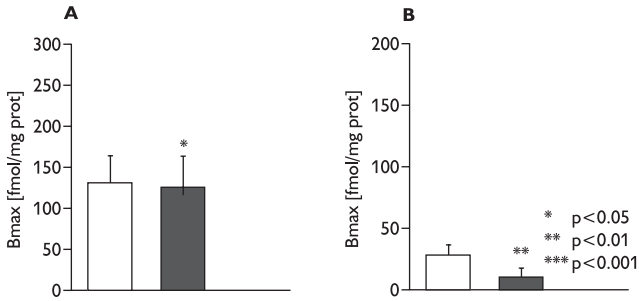

Figure 4 – DA receptor density in the striatum of Lurcher mutants and wild type mice. A: D_1-like receptor; B: D_2 -like receptor. Wild type □; Lurcher ■

with findings that activity of dopamine transporter (uptake sites) was similar to controls, except for a decrease in the subthalamic nucleus [15]. This data strengthen the hypothesis about the main role of dopamine receptors in coping with changed condition in olivocerebellar degeneration. Similarly to that finding, no decrease was found for aspartate, gamma-aminobutyric acid (GABA), glycine, as well as dopamine and its metabolites [16]. Once again, this could be supportive finding for hypothesis that receptor changes are the most important events in coping with mutation in GluRδ2.

Similar to our results with spatial learning, in Lurcher, there was an improvement in the distance travelled on the suspended horizontal string after dextromethorphan (an NMDA antagonist) and L-dopa/carbidopa, but not after SKF 77434 [17].

On the other hand, in the Purkinje Cell Degeneration (Nna1pcd, pcd) mutant mouse, dopamine transporters [18] were higher compared with wild-type mice in the ventral mesencephalic dopaminergic nuclei and in the lateral striatum, motor cortex, septum and in the deep cerebellar nuclei, but they were significantly lower in the molecular layer. The D1-like receptors were significantly higher in substantia nigra. The D2/D3 receptors exhibited a significant decrease in lateral divisions of

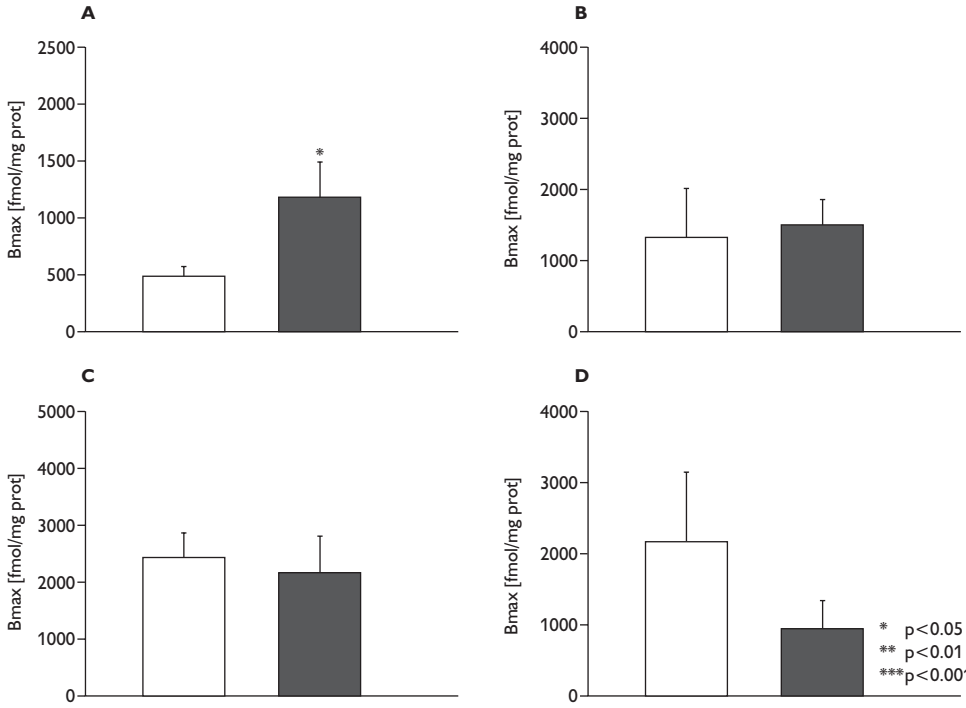

Figure 5 – DA receptor density in the hippocampus and striatum of Lurcher mutants and wild type C3H mice. A: D_1 like receptor, hippocampus; B: D_2 like receptor, hippocampus; C: D_1-like receptor, striatum, D_2-like receptor. Wild type □; Lurcher ■

the striatum. Significant increases in D2-like receptors were observed in most divisions of the striatum as well as in septum, hippocampus, and piriform cortex. In the cerebellum of Nna1pcd mice, D2-like receptors were significantly decreased in the molecular layer.

These results provide information about the role of dopamine transmitter – receptor system in mutants with olivocerebellar degeneration, especially when we compare these results with our findings in mice of another strain (C3H).

Taken together, it is possible to conclude that dopaminergic system plays an important role in mice with olivocerebellar degeneration and that dopamine receptors are, in contrast to dopamine transporters, subject of changes in these mutants. Moreover, our data reveal that dopamine receptor system is preserved in Lurchers as shown by similar changes in spatial learning after D1-dopamine antagonist SCH23390.

Conclusions

a) the different results of spatial learning in neurodefective Lurcher mutants and wild type mice confirm the participation of the cerebellum in this behavioral activity
b) the possibility of influencing spatial learning mainly by means of D_1 DA receptor antagonist in both types of mice proves the role of dopaminergic system in this cognitive process regardless of the cerebellum
c) significantly higher density of D_1 and D_2 DA receptors in Lurcher mutants' hippocampus compared to wild type mice can be in relationship with differences in spatial learning between animals of both types of mice
d) significantly lower density of D_1 DA receptors in the cerebellum of Lurcher mutants can be connected to other functional differences between them and wild type mice
e) insignificant differences of D_1 and D_2 DA receptors density in the striatum between Lurcher mutant and wild type mice give evidence that impaired motor functions in Lurchers are not caused by changes of the dopaminergic transmission in this structure.

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References

1. YUZAKI M.: The delta2 glutamate receptor: a key molecule controlling synaptic plasticity and structure in Purkinje cells. *Cerebellum.* 3: 89–93, 2004
2. CADDY K. W. T., VOŽEH F.: The effect of 3-acetylpyridine on inferior olivary neuron degeneration in Lurcher mutant and wild type mice. *Eur. J. Pharm.* 330: 139–142, 1997.
3. VOŽEH F., CENDELÍN J., MOTÁŇOVÁ A.: The development of different types of learning in cerebellar degeneration model. *Homeostasis* 39: 248–250, 1999.
4. KŘÍŽKOVÁ A., VOŽEH F.: Development of early learning and topical motor skills in a model cerebellar degeneration. *Behav. Brain Res.* 150: 65–72, 2004.

5. CADDY K. W. T., BISCOE T. J.: Structural and quantitative studies on the normal C3H and Lurcher mutant mouse. *Phil. Trans. R. Soc. London (Biol.)* 287: 167–201, 1979.
6. ZUO J., DE JAGER P. L., TAKAHASHI K. A., JANG W., LINDEN D. J., HEINTZ N.: Neurodegeneration in Lurcher mice cause by mutation in δ2 glutamate receptor gene. *Nature* 388: 769–773, 1997
7. HANTRAYE P.: Modeling dopamine system dysfunction in experimental animals. *Nucl. Med. Biol.* 25: 721–728, 1998.
8. MYSLIVECEK J.: Inhibitory learning and memory in newborn rats. *Prog Neurobiol.* 53: 399–430, 1997.
9. JAY T. M., ROCHER C., HOTTE M., NAUDON I., SPEDDING M.: Plasticity at hippocampal to prefrontal cortsx synapses is impaired by loss of dopamine and stress: importance for psychiatric diseases. *Neurotox. Res.* 6: 233–244, 2004.
10. DEVOINO L., IDOVA G., ALPERINA E., CHEIDO M.: Brain neuromediator systems in the immune response control: pharmacological analysis of pre – and postsynaptic mechanisms. *Brain Res.* 633: 267–74, 1994.
11. IDOVA G. V., CHEIDO M. A., ZHUKOVA E. N., DEVOINE L. V.: Stimulation of the immune response during activation of the dopaminergic system in mice with opposite types of behavior. *Neurosci. Behav. Physiol*. 34: 417–421, 2004.
12. VOŽEH F., CENDELÍN J., ŠÍMA P., VIRTOVÁ M.: The effect of influencing dopaminergic system on some nervous and immune characteristics in Lurcher mutant mice. *Homeostasis* 43: 195–198, 2005.
13. MORRIS R.G. M.: Development of a water–maze procedure for studying spatial learning in the rat. *J. Neurosci. Meth. 11*: 47–64, 1984.
14. BENEŠ J., NOVÁKOVÁ M., KVETŇANSKÝ R., MYSLIVEČEK J.: Developmental changes of some G-protein coupled receptors affected by c-fos knock-out. *Prague Med. Rep*. 107: 61–70, 2006.
15. STRAZIELLE C., LALONDE R., AMDISS F., BOTEZ M. I., HEBERT C., READER T. A.: Distribution of dopamine transporters in basal ganglia of cerebellar ataxic mice by [125I]RTI-121 quantitative autoradiography. *Neurochem. Int.* 32: 61–68, 1998.
16. READER T. A., STRAZIELLE C., BOTEZ M. I., LALONDE R.: Brain dopamine and amino acid concentrations in Lurcher mutant mice. *Brain Res. Bull*. 45: 489–493, 1998.
17. THULLIER F., LALONDE R., LESTIENNE F.: Effects of dopaminergic agents and of an NMDA receptor antagonist on motor coordination in Lurcher mutant mice. *Pharmacol. Biochem. Behav.* 63: 213–219, 1999.
18. DELIS F., MITSACOS A., GIOMPRES P.: Dopamine receptor and transporter levels are altered in the brain of Purkinje Cell Degeneration mutant mice. *Neuroscience* 125: 255–268, 2004.