Glutamate Receptor Block in Lurcher Mutant Mice during Ontogeny and its Effect on Hippocampal Long-term Potentiation

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Abstract: Basic evaluation of the effect of chronic NMDA glutamate receptor (NMDAR) blockade on the hippocampal long-term potentiation (LTP) was performed in an animal model of inborn olivo-cerebellar degeneration (Lurcher mutant mice, LMM). NMDA receptor antagonist MK-801 was administered to mice in the dose 0.2 mg/kg of body weight, daily during two periods of their ontogeny: D5–D26 and D91–D111. In the consecutive 15 days some behavioral characteristics were studied using special methods for physical activity testing. Then LTP was investigated in LMM and also in their healthy littermates which served as controls (wild-type, WT). LTP in animals pre-treated with MK-801 showed significant long-term suppression of NMDAR activity, in both WT and LMM despite certain small differences between them. Our results show that cerebellar pathology on one hand and a physical activity on the other hand can influence the LTP in hippocampal region. It can be concluded that the results support the ideas of close functional cooperation between the brain structures which are involved in mechanisms of learning and memory.

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Introduction

Lurcher mutant mice (LMM) represent a natural model of genetically determined olivocerebellar degeneration (Zuo et al., 1997). Heterozygote individuals (+/Lc) are characterized by the postnatal complete loss of cerebellar Purkinje cells (excitotoxic apoptosis) and by the decrease of number of granule cells and inferior olivary neurons (secondary to the loss of Purkinje cells). Affected homozygots (Lc/Lc) are not viable. Unaffected homozygots, wild type mice (+/+) are completely healthy and serve as controls.

Recent experimental studies (including ours) suggest that in LMM some cognitive functions are changed, especially in development of spatial learning in Lc/+ and +/+ during early postnatal period of life using the Morris water maze (Vožeh et al., 1999). Also changes in the area of motor learning examination were described (Křížková and Vožeh, 2004). Some neurons of Lurchers are more sensitive to neurotoxic substances (Caddy and Vožeh, 1997) and other experiments discovered a higher degree of excitability of the CNS in Lc/+ when compared with +/+ using a method of audiogennic epilepsy (Cendelín and Vožeh, 1999). Similar findings were obtained in experiments which measured brain cortical activity after previous electrical and drug stimulation (Barcal et al., 2000; Sobotka et al., 2000). Differences in hippocampal activity (LTP) were found in anesthetized Lc/+ in comparison with +/+ (Barcal et al., 2001).

Neurodegeneration in LMM is primarily caused by a mutation in the gene expression for the delta-2 glutamate receptor subunit. Delta receptors (GluRδ) were classified as special kind of ionotropic glutamate receptors but their properties and function are not yet fully understood. Recent experimental results suggest that GluRδ are localized in the cerebellum, auditory and vestibular system (Yuzaki, 2003). Possible influencing of excitotoxic mechanisms is the main reason for studying of pharmacological interactions between glutamergic system and many active substances and drugs.

N-methyl-D-aspartate receptors (NMDAR) are members of the ionotropic glutamate receptor family. They mediate most of the excitatory synaptic transmission in the mammalian central nervous system (CNS). The physiological function of NMDAR was undoubtedly confirmed for neuronal survival, differentiation and synaptic plasticity. An increase of NMDAR activity also leads to long-term changes in neuronal functions including protein kinase cascades as well as modulation of transcription factors (Malenka and Bear, 2004); these changes suggest that NMDAR function is also important in mechanisms of learning and memory at the cellular level (Collingridge, 2003).

In the present study, the effect of long-term NMDAR blockade on the hippocampal long-term potentiation (LTP) during two periods of postnatal life was studied.

Material and Methods

We used 53 mice of both sexes (strain B6CBA), Lurcher mutant mice (n=31) and wild type (n=22). All animals were housed under standard conditions, i.e. 12/12

hours light (6:00 am – 6:00 pm) /dark period and food and water were available *ad libitum*. All experiments were performed in full agreement with the EU Guidelines for scientific experimentation on animals and with the permission of the Ethical Commission of the Faculty of Medicine in Plzeň.

MK-801 (dizocilpin), highly potent non-competitive NMDA receptor antagonist, was administered intraperitoneally (i.p.) in dose 0.2 mg/kg of body weight, daily during two periods of ontogeny: D5–D26 and D91–D111. Saline solution in an equivalent dose was administered i.p. as control in the same experimental time design. Thus eight groups of animals were used for final statistical evaluation.

During consecutive 15 days some behavioral characteristics were studied using special methods (open field, motor learning examination and spatial navigation test in Morris water maze). After that the hippocampal long-term potentiation (LTP) was performed. All LTP procedures were done as acute experiments under urethane anaesthesia (20% solution in dose 1.5 g/kg of body weight i.p.). After the loss of nociceptive and corneal reflexes the animal was fixed into the stereotaxic frame. Body temperature was measured by rectal probe and small heating pad (Fine Science Tools, USA) was used for temperature keeping (37 °C ± 0.5). Then a surgical preparation and calva cleaning were done. Using high-speed microdrill the corresponding holes were prepared; for stimulation in perforant path: (AP – λ, L – 3.0, V – 2.0) and registration in ipsilateral hilus of dentate gyrus (AP – 2.0, L – 1.7, V – 1.9) stainless steel electrodes were used. Grounding electrode was fixed in contralateral prefrontal area to the bone with a screw. All calculation has been done according to the bregma point (Franklin and Paxinos, 1997). For the basal low frequency (LFS), 16 biphasic pulses 2–4 V, 0.1 Hz, duration 0.1 ms, for high-frequency stimulation (HFS) 100 Hz, 3 bursts each 15 s were applied.

Experimental protocol consisted of three parts:

1st – registration of basal response (then used as average value from 3 responses after LFS – 100%);
2nd – high-frequency (tetanic) stimulation (HFS);
3rd – registration of responses after HFS – time intervals 5th, 10th, 15th, 20th, 30th, 45th and 60th min.

Because obtained data did not show normal distribution (verified with Kolmogorov-Smirnov test) the nonparametric tests were used: Kruscal-Wallis test using the amplitude of population spike for comparison and post-hoc Mann-Whitney test ($p<0.05$ was accepted as level of significance).

Results

Our results confirmed the ability of the generating of LTP in animals of both groups i.e. wild-type (healthy controls) and Lurcher mutant mice. A significant difference was revealed between wild-type mice influenced by MK-801 during the fourth month of postnatal life and control animals (pre-treated with saline); a suppression

of LTP during all time of measurement after administration of NMDA blocker was depicted (Figure 1, Table 1).

A comparison of wild-type mice influenced by MK-801 during early postnatal period with corresponding control animals showed lower level of LTP blockade with significant differences only in 15th and 20th minutes (Figure 2, Table 2).

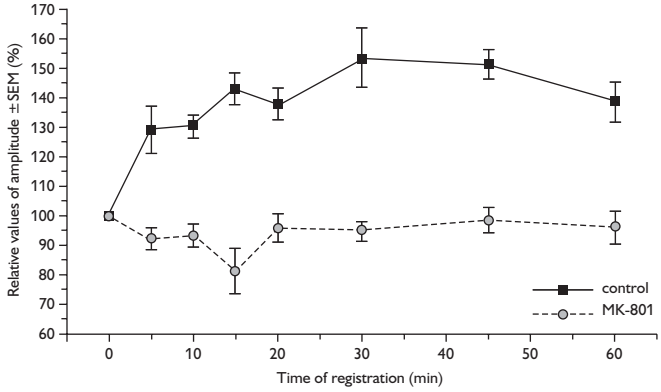

Figure 1 – Comparison of relative values of population spike amplitude ± SEM between unaffected (with saline administration) wild-type animals (controls, squares) and wild-type animals pre-treated with MK-801 (circles) during D91–D111 (adults).

Table 1 – Post-hoc Mann-Whitney test during seven intervals of measurement

min	5	10	15	20	30	45	60
p	**0.0027**	**0.0027**	**0.0027**	**0.0027**	**0.0027**	**0.0027**	**0.0027**

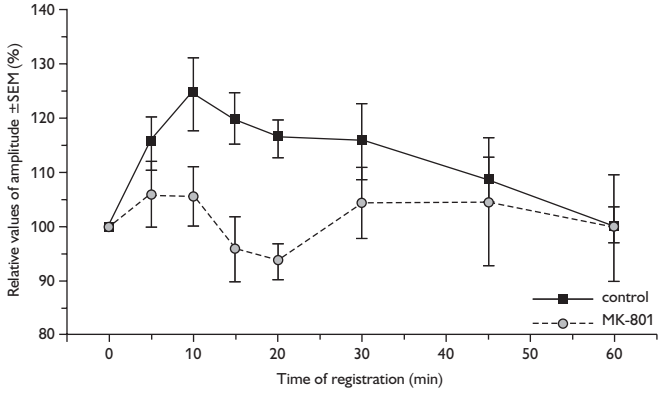

Figure 2 – Comparison of relative values of population spike amplitude ± SEM between unaffected (with saline administration) wild-type animals (controls, squares) and wild type mice with MK-801 pre-treatment (circles) during D5–D26 (young).

Table 2 – Post-hoc Mann-Whitney test during seven intervals of measurement

min	5	10	15	20	30	45	60
p	0.27332	0.08284	**0.01762**	**0.0062**	0.64808	0.85513	0.85513

In adult Lurcher mutant mice the level of LTP is generally lower (compared with wild-type mice) but also differences between animals pre-treated with MK-801 and control animals were shown (Figure 3, Table 3).

A different situation was depicted in young LMM. Control animals (pre-treated with saline) revealed clear LTP during all measuring intervals. MK-801

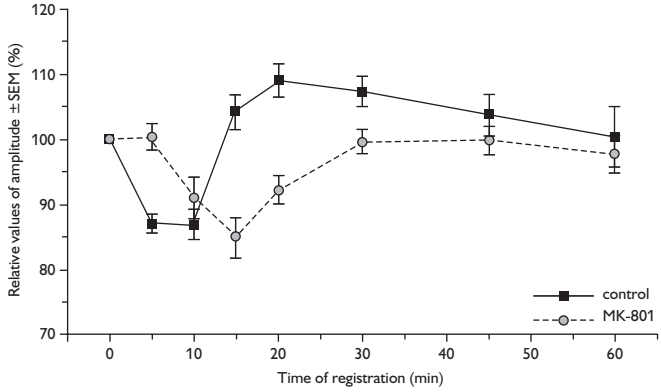

Figure 3 – Comparison of relative values of population spike amplitude ± SEM between unaffected (with saline administration) Lurcher mutant mice (controls, squares) and Lurcher mutant mice pre-treated with MK-801 (circles) during D91–D111 (adults).

Table 3 – Post-hoc Mann-Whitney test during seven intervals of measurement

min	5	10	15	20	30	45	60
p	**0.00218**	0.39295	**0.00257**	**0.00303**	**0.02703**	0.31488	0.61530

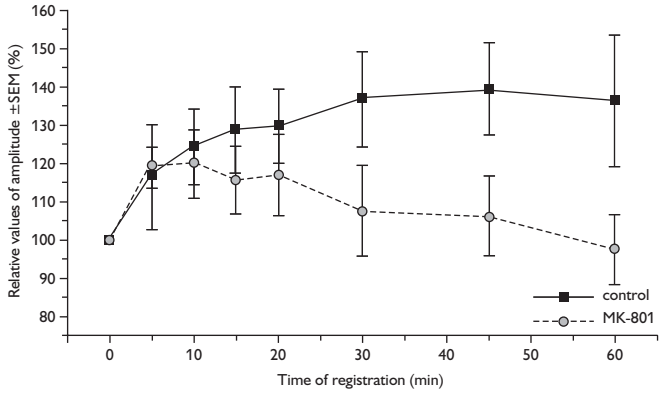

Figure 4 – Comparison of relative values of population spike amplitude ± SEM between unaffected (with saline administration) Lurcher mutant mice (controls, squares) and Lurcher mutant mice pre-treated with MK-801 (circles) during D5–D26 (young).

Table 4 – Post-hoc Mann-Whitney test during seven intervals of measurement

min	5	10	15	20	30	45	60
p	0.41131	0.71500	1.0	0.52282	0.06789	**0.044611**	**0.04461**

administration suppressed their LTP ability but only in last two time intervals (45th resp. 60th min) significantly (Figure 4, Table 4).

Discussion

NMDA receptors are revealed as important part of different neurophysiological mechanisms like stressful conditions (Yamane et al., 2008), appetitive conditioning (Ithak, 2008), pain perception (Chizh, 2007; Liu et al., 2008) or excitotoxic pathway during cerebral ischemia and stroke (András et al., 2007). Some animal models of neuropsychiatric disorders are based on the manipulation with NMDAR especially in case of schizophrenia (Nabeshima et al., 2006).

In recent studies in rodents, NMDAR blockade impaired learning and memory by the disruption of LTP, especially spatial navigation which is hippocampal-dependent (Morris, 2003). Some studies in humans suggest that block of NMDAR impairs encoding (i.e. mechanism of learning), but not retrieval (i.e. memory); similar results were shown both in spatial and nonspatial information processes (Rowland et al., 2005). Administration of MK-801 blocks possible secondary enzymatic activation like calmodulinkinases, proteinkinases and nitric-oxide synthases pathways which is a secondary response during the first stage of postsynaptic changes (i.e. activation of AMPA channels which allows opening of NMDA channels and Ca^{2+} influx increases and higher intraneuronal concentration of Ca^{2+} leads to the serious consequences in the neuronal activity (Bellone and Nicoll, 2007). Second part of LTP induction is localized at the presynaptic level where nitric oxide, arachidonic acid and probably CO may act as retrograde messengers and modify the final effect. These messengers (or neuromodulators) reach the presynaptic nerve terminal by diffusion, where they enhance transmitter releasing into the synaptic cleft (Hölscher, 1999).

Results from our previous study focussed on the acute (i.e. single-dose) administration of MK-801 and its effect on the LTP showed that the period of effective NMDA receptor blockade is not permanent (Barcal et al., 2007). Long-term (repetitive) administration of MK-801 during 20 days of postnatal life suggests strong suppression of NMDAR activity which leads to the decrease of hippocampal LTP. Possible positive effect of physical activity on the LTP level especially in LMM is in the full agreement with papers where the clear improvement of spatial learning ability was described after similar procedures (Cendelín et al., 2008) and during stay in enriched environment (Caston et al., 1999).

Conclusion

MK-801 pre-treatment caused a clear suppression of LTP (i.e. magnitude of spikes amplitude). In control young Lurcher mutant mice (with incomplete neuronal loss) a relatively higher LTP ability (compared with adults) was described.

We can finally conclude that cerebellar pathology on one hand and a physical activity on the other hand can influence the “level” or “intensity” of NMDA

receptor blockade and LTP in hippocampal region, too. Taken together, the results support the idea of close functional cooperation between the brain structures which are involved in mechanisms of learning and memory (the cerebellum and hippocampus).

References

András, I. E., Deli, M. A., Veselka, S., Hayashi, K., Hennig, B., Toborek, M. (2007) The NMDA and AMPA/KA receptors are involved in glutamate-induced alterations of occludin expression and phosphorylation in brain endothelial cells. *J. Cereb. Blood Flow Metab.* **27**, 1431–1443.

Barcal, J., Ježek, K., Vožeh, F., Žalud, V. (2000) Changes of excitability in the cerebellar degeneration model (Lurcher mutant mice). *Physiol. Res.* **49**, P38.

Barcal, J., Vožeh, F., Žalud, V. (2001) The differential cortical and hippocampal activity in the cerebellar degeneration model. *Physiol. Res.* **50**, P2.

Barcal, J., Korelusová, I., Cendelín, J., Vožeh, F. (2007) The time-dependent block of NMDA glutamate receptor influences hippocampal LTP in inborn cerebellar degeneration mouse model. *Prague Med. Rep.* **108**, 29–36.

Bellone, C., Nicoll, R. A. (2007) Rapid bidirectional switching of synaptic NMDA receptors. *Neuron* **55**, 779–785.

Caddy, K. W. T., Vožeh, F. (1997) The effect of 3-acetylpyridine on inferior olivary neuron degeneration in Lurcher mutant and wild type mice. *Eur. J. Pharmacol.* **330**, 139–142.

Caston, J., Devulder, B., Jouen, F., Laponce, R., Delhaye-Bouchard, N., Mariani, J. (1999) Role of an enriched environment on the restoration of behavioral deficits in Lurcher mutant mice. *Dev. Psychobiol.* **35**, 291–303.

Cendelín, J., Vožeh, F. (1999) Assessment of CNS excitability in natural model of cerebellar degeneration. *Homeostasis* **39**, 115–116.

Cendelín, J., Korelusová, I., Vožeh, F. (2008) The effect of repeated rotarod training on motor skills and spatial learning ability in Lurcher mutant mice. *Behav. Brain Res.* **189**, 65–74.

Chizh, B. A. (2007) Low dose ketamine: A therapeutic and research tool to explore N-methyl-D-aspartate (NMDA) receptor-mediated plasticity in pain pathways. *J. Psychopharmacol.* **21**, 259–271.

Collingridge, G. L. (2003) The induction of N-methyl-D-aspartate receptor-dependent long-term potentiation. *Philos. Trans. R. Soc. Lond. B* **358**, 635–641.

Franklin, K. B. J., Paxinos, G. (1997) *The Mouse Brain in Stereotaxic Coordinates*. Academic Press, San Diego.

Hölscher, C. (1999) Synaptic plasticity and learning and memory: LTP and beyond. *J. Neurosci. Res.* **58**, 62–75.

Ithak, Y. (2008) Role of the NMDA receptor and nitric oxide in memory reconsolidation of cocaine-induced conditioned place preference in mice. *Ann. N. Y. Acad. Sci.* **1138**, 350–357.

Křížková, A., Vožeh, F. (2004) Development of early motor learning and topical motor skills in a model of cerebellar degeneration. *Behav. Brain Res.* **150**, 65–72.

Liu, X. J., Gingrich, J. R., Vargas-Caballero, M., Dong, Y. N., Sengar, A., Beggs, S., Wang, S. H., Ding, H. K., Frankland, P. W., Salter, M. W. (2008) Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex. *Nat. Med.* **14**, 1325–1332.

Malenka, R. C., Bear, M. F. (2004) LTP and LTD: An embarrassment of riches. *Neuron* **44**, 5–21.

Morris, R. G. M. (2003) Long-term potentiation and memory. *Philos. Trans. R. Soc. Lond. B* **358**, 643–647.

Nabeshima, T., Mouri, A., Kurzi, R., Noda, Y. (2006) Dysfunction of NMDA receptor-signaling in mice following withdrawal from repeated administration of phencyclidine. *Ann. N. Y. Acad. Sci.* **1086**, 160–168.

Rowland, L. M., Astur, R. S., Jung, R. E., Bustillo, J. R., Lauriello, J., Yeo, R. A. (2005) Selective cognitive impairments associated with NMDA receptor blockade in humans. *Neuropsychopharmacology* **30**, 633–639.

Sobotka, P., Barcal, J., Žalud, V., Vožeh, F. (2000) The effect of caffeine on the heart activity of mice with inborn cerebellar degeneration. *Homeostasis* **40**, 128–129.

Vožeh, F., Cendelín, J., Motáňová, A. (1999) The development of different types of learning in cerebellar degeneration model. *Homeostasis* **39**, 248–250.

Yamane, H., Tsuneyoshi, Y., Denbow, D. M., Furuse, M. (2008) N-methyl-D-aspartate and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors involved in the induction of sedative effects under acute stress in neonatal chicks. *Amino Acids* **37(4)**, 733–739.

Yuzaki, M. (2003) The delta2 glutamate receptor: 10 years later. *Neurosci. Res.* **46**, 11–22.

Zuo, J., De Jager, P. L., Takahashi, K. J., Juany, W., Linden, D. J., Heintz, N. (1997) Neurodegeneration in Lurcher mice caused by mutation in δ2 glutamate receptor gene. *Nature* **388**, 769–773.