Does Transplantation of Cerebellar Embryonic Tissue Influence Hippocampal LTP in Adult Lurcher Mutant Mice?

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Abstract: Possible influence of embryonic cerebellar graft transplanted into the adult neurodegenerative brain in Lurcher mutant mice on long-term potentiation (LTP) in hippocampus was investigated. Evaluation of LTP ability and comparison with the tests of motor learning suggests similarities between magnitude of LTP and criteria of motor learning. Also interstrain differences were described. Our results support ideas about tight cooperation among brain structures which are involved in mechanisms of learning and memory.

Introduction

Long-term potentiation (LTP) is revealed as generally accepted electrophysiological model of learning and memory [1]. The phenomenon of LTP was first described by Terje Lømo in Oslo [2]. His work was focussed on the phenomenon of frequency potentiation, an increase in the magnitude of responsiveness of cells following a series of rapidly applied activations. Lømo found that repetitive high-frequency electrical stimulation (tetanus) of one pathway resulted in a steeper rise time (slope) of the excitatory synaptic potentials to a subsequent single pulse. He also observed that following a tetanus there was recruitment of a greater number of cells reaching the threshold for an action potential, reflected in a greater „population spike". These tetanus-induced changes in synaptic and cellular responses to single pulses lasted for several hours. During last three decades some fundamental properties that make LTP such an attractive model of memory were identified (i.e. description of hippocampal regions, its temporal characteristics, specificity and associativness) [3].

In past years a key role of different kinds of glutamate receptors in the ability to form long-term potentiation (LTP) was established [4]. Two major types of glutamate receptors are involved in this mechanism of synaptic changes which serves as cellular model of learning: NMDA type, N-methyl-D-aspartic acid, and AMPA/kainate type, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid. NMDA receptor type is widely distributed in mammalian CNS, namely in the hippocampus and cerebral cortex. It was recognized as a slow component in the generation of EPSP and it acts also in various mechanisms of synaptic plasticity. On the other hand, overexpression of glutamate receptors (under some pathological conditions such as brain ischemia or traumatic injury) leads to the excitotoxic brain damage with accelerated apoptotic death of neurons.

An important example of inborn pathology of glutamatergic transmission is delta-2 receptor gene mutation; this receptor is preferably expressed by cerebellar Purkinje cells. Pathological overexpression leads to the activation of excitotoxic apoptotic mechanisms which are selective and cell-autonomous. During first postnatal three months, Purkinje cells are lost and the density of granule cells and inferior olivary neurons dramatically decreased secondarily [5]. According to this pathogenetical mechanism Lurcher mutants mice (LMM)

represent an excellent model for studying the excitotoxic cell damage, death and other consequences of this pathology.

In past years we published recent findings which suggest a close cooperation and possible interaction between cerebellar and hippocampal structures. Disturbance of motor learning in LMM resulting from cerebellar injury at one side [6] is related to changes in the hippocampal region including LTP [7].

In this paper a possible influence of cerebellar embryonic graft transplanted in adult Lurcher mutant mice on the hippocampal LTP induction was studied.

Materials and methods

Experiments were done in two different strains of Lurcher mutant mice (LMM, heterozygotes +/Lc). In +/Lc animals of both strains have the mutation of the same gene with the same type of resulting neurodegeneration. The strain C3H is usually characterised by light grey-coloured skin in +/Lc mice and dark grey-coloured skin in wild type (+/+) – healthy littermates. In the strain C57B1/7 the black or dark grey-coloured types in both +/Lc and +/+ individuals are present.

We used adult (5–18 month) Lurcher mutant mice (n=20, both strains, body weight 19–32 g). All manipulations and used methods were performed in full agreement with the „EU Guidelines for scientific experimentation on animals".

A. Transplantation

Embryonic cerebellar tissue was obtained from 12 to 13-day-old mice without the Lurcher mutation. Pregnant donor females were euthanized by overdosing with Thiopental at gestation day 12 or 13. The embryos were removed from the uterus and pooled in the cold aqueous solution of 0.9% natrium chloride and 0.6% glucose. Their brainstems were isolated and cerebella were dissected and pooled in the same solution. Adult Lurcher mutant mice of the C3H and C57Bl/7 strains were used as the host. Animals older than 60 day were considered adult. The donor mouse was anaesthetized with intraperitoneal application of combination of Ketamine (100 mg/kg b.w.) and Xylazine (16 mg/kg b.w.). The mouse was fixed in a stereotaxic holder. Soft tissue of the occipital area of the head was cut in the midline and a hole (2 mm in diameter) was drilled in the middle of the occipital bone. Two solid pieces of the embryonic cerebellum (tissue obtained from one embryo) and 10 μl of vehiculum (aqueous solution of 0.9% natrium chloride and 0.6% glucose) were injected with a glass microcapillary into the host cerebellum. Finally, the wound was sutured in one layer. Sham-operated control animals received only vehiculum during the same procedure [8].

Table 1 – Time scale of experiments

	Transplantation				Motor learning tests			LTP		
weeks	4	8	12	16	20	24	28	32	36	40

B. Tests of motor learning

Motor coordination was investigated with a set of three tests: horizontal bar, ladder and rotarod [6]. All tests were repeated four times in one run. We evaluated mean percentage of successful trials in mice with histologically proven graft presence, in mice in which the graft disappeared and in sham-operated controls. The criterion of successful trial was to stay on the apparatus for 60 s or to leave it actively. For statistical analysis Man-Whitney test was used. In young mice motor functions were examined 3 weeks after the surgery, in adult animals before and then 4, 8 and 10 weeks after the surgery.

C. Hippocampal LTP

Hippocampal long-term potentiation was performed as acute experiments under urethane anesthesia (20%, 1,5 g/kg b.w., intraperinoneally). 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 (Fine Science Tools, USA) 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 screw. All calculation has been done according to the bregma point [9].

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 – tetanus of high-frequency (HFS); 3rd – registration of responses after HFS – time intervals 5th, 10th, 15th., 20th, 30th, 45th and 60th min.

The final statistical evaluation was performed by the ANOVA test using the amplitude characteristics as comparable parameters.

Results

Both strains of control LMM (i.e. without transplantation) revealed similar ability to produce the hippocampal LTP. The increase of magnitude of EPSP amplitude was long-lasting and suggests a permanent change at the synaptic level. Very important and also controversial results were obtained from comparison of animals with embryonic graft in both strains (Figures 1 and 2). In C3H strain, LMM with transplanted embryonic tissue had statistically significant enhancement of EPSP amplitude in comparison with controls (Figure 1, ANOVA: $p < 0.001$). On the other hand, C57Bl/7 mice revealed opposite changes, i.e. the LTP in transplanted animals was significantly worse and practically completely blocked. (Figure 2,

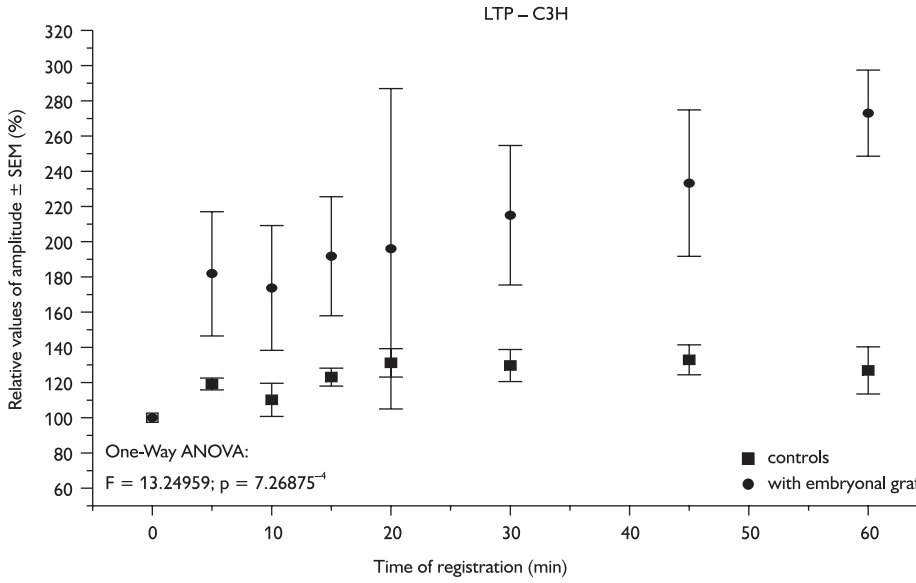

Figure 1 – Comparison of relative values of spike amplitude (in %) ± SEM in control animals (squares) and transplanted mice (circles); C3H strain.

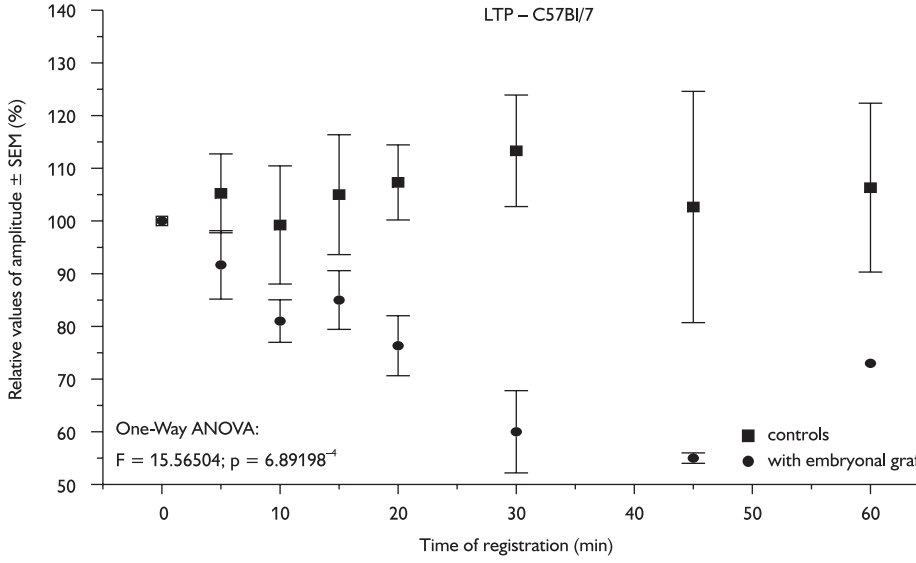

Figure 2 – Comparison of relative values of spike amplitude (in %) ± SEM in control animals (squares) and transplanted mice (circles); C57Bl/7 strain.

ANOVA: $p < 0.001$). Results of motor learning tests performed before LTP showed similarities with the LTP. In C3H mice a trend of enhancement of motor skills in animals with embryonic graft was depicted (Figure 3) together with statistically significant increase of successful meeting criteria in 6th week. C57Bl/7 mice revealed better results in control animals during all weeks but differences were not significant (Figure 4).

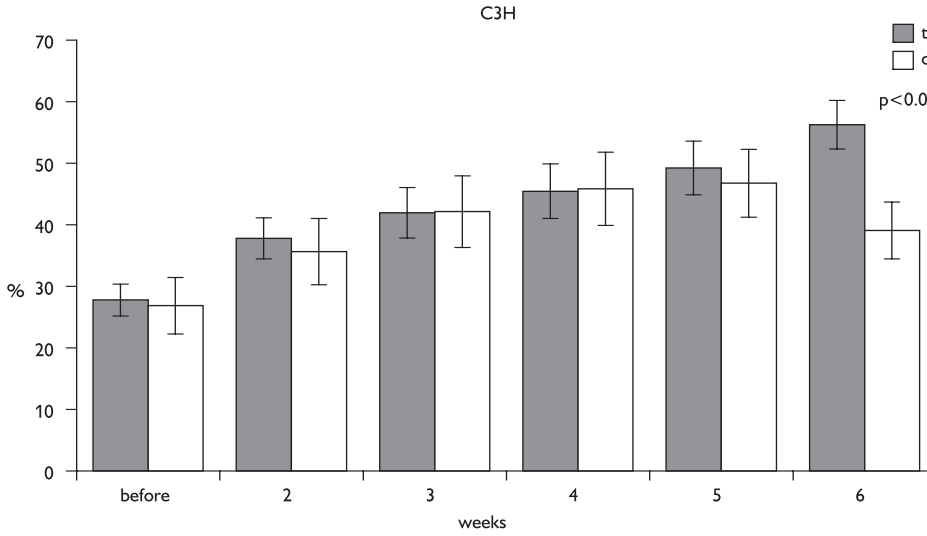

Figure 3 – Percentage of successful trials in all three motor tests before and after transplantation; C3H strain.

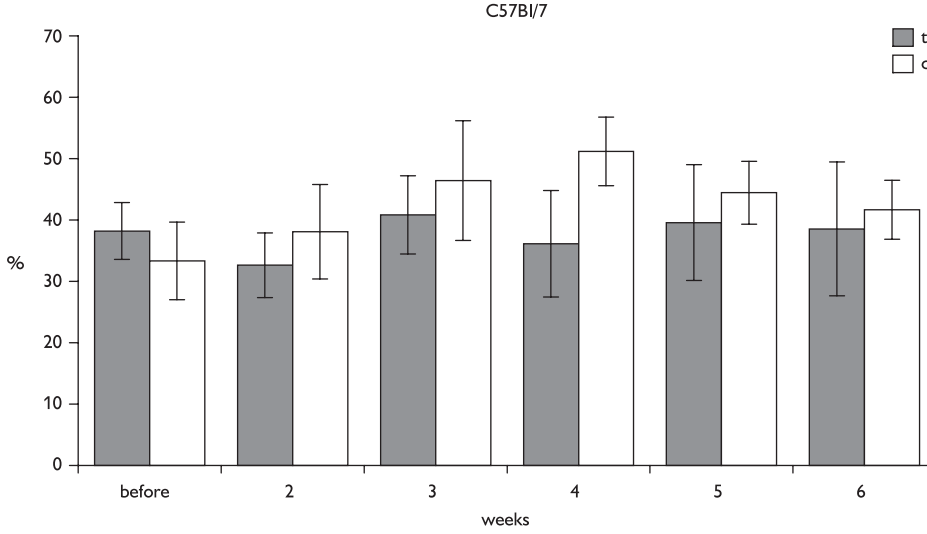

Figure 4 – Percentage of successful trials in all three motor tests before and after transplantation; C57Bl/7 strain.

Discussion

From many previous studies we know that LTP mechanism includes two main parts. First is postsynaptic depolarization via activation AMPA and/or kainate channels which allows opening of NMDA channels. Ca^{2+} influx increases and higher intraneuronal concentration of Ca^{2+} causes a number of secondary effects. Second part of LTP induction is localized at the presynaptic level. The retrograde messengers (nitric oxide, arachidonic acid and probably CO) are formed as result of Ca^{2+} increase after the NMDA receptor activation. These messengers (or neuromodulators) reach the presynaptic nerve terminal by diffusion, where they enhance transmitter release to the synaptic cleft [10].

A final effect of Ca^{2+} increase in postsynaptic segment is the activation of signalling substances such as protein kinase C, p42/44 mitogen-activated protein kinase (ERK), and Ca^{2+}/calmodulin-dependent kinases. Next step leads to phosphorylation of the transcription factor cAMP-response element binding protein (CREB) which can start transcription of immediate early genes (third messengers) required for LTP. Thus later phases of LTP are dependent on gene transcription and protein synthesis [11].

Recent studies suggest that brain-derived neurotrophic factor (BDNF) has similar effects as NMDA receptor stimulation and BDNF induces LTP [12]. As the same result of NMDA receptor stimulation, ERK and CREB are also activated by BDNF [13]. One of possible explanation of the embryonic graft positive effect on the LTP (in C3H strain) may be the fact that embryonic cerebellar tissue contains higher amount of BDNF which can act not only in place of transplantation (ie. in cerebellar area) but also in hippocampal regions. The finding of significant interstrain difference (i.e. LTP blockade in C57Bl/7 strain) remains unclear and other similar experiments are needed.

Our previous study demonstrated that primary cerebellar pathology, i.e. mutation of delta-2 glutamate receptor gene may have a possible secondary influence on the level of NMDA receptor blockade caused by MK-801 [14]. We expected, in accordance with these results, that ability to create higher LTP production on the one side and higher spatial navigation ability on the other one were not identical. These findings strongly support idea that the mechanism of spatial learning (i.e. hippocampal LTP at the synaptic level) involves a number of nonspatial components (motor control or stress factors) that are not hippocampus dependent and can influence final results [15].

Conclusion

Taken together, an embryonic graft influences LTP in dentate gyrus of host animal – adult Lurcher mutant mice. Trend of LTP enhancement in transplanted animals of C3H strain correlates with the tests of motor

learning (which are not primary dependent on hippocampal function). LTP blockade in transplanted animals of the C57Bl/7 strain correlates with the tests of motor learning too, LTP in transplanted animals was impaired. Statistically significant differences between transplanted and control animals of both strains suggest possible impact of trophic factors originated in the embryonic tissue.

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