

# Hippocampal Long-term Potentiation in Adult Lurcher Mutant Mice: Effect of Embryonic Cerebellar Graft and Motor Training

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**Abstract:** Possible effect of trophic factors from embryonic cerebellar graft transplanted in adult Lurcher mutant mice on LTP as electrophysiological marker of learning and memory process was studied. Also the combination of the transplantation and long-term forced motor training was investigated. An evaluation of LTP ability in four animal groups (transplanted, sham-operated, with and without forced motor activity) and comparison among them showed the highest LTP improvement in the group with combination of both influences (ie. transplantation and motor training).

## Introduction

The cerebellum is an important structure for the regulation of motor function [1, 2] and also for the motor learning [3]. Generally, the motor learning capabilities are decreased after cerebellar injury and after lesion of the olivocerebellar pathway caused by 3-acetylpyridine used as a selective toxin [4]. Motor skills and motor learning are altered in various mutant mice exhibiting cerebellar degeneration, such as weaver [5], staggerer [6] and Lurcher [7, 8, 9]. Heterozygous Lurcher mutant mice (+/Lc) have been widely investigated. The primary cause – Lurcher mutation – is located in the glutamate receptor delta 2 subunit, leading to an increase of the  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  currents, and to Purkinje cells apoptosis caused by excitotoxic glutamate overexpression [10]. Animals are ataxic [11, 12, 13] and motor learning usually tested on the rotarod is delayed. Their problems are both due to a precocious degeneration of the Purkinje cells of the cerebellar cortex, which begins at the end of the first postnatal week and which is almost 100% by two months of age, and a retrograde degeneration of most granule cells (90%) and of the inferior olive neurons (60–75 %) [14, 15, 16]. One of possible methods which may partially repair this serious motor impairment is a transplantation of embryonal cerebellar tissue. Beside clear direct effect on the development of new neural connections in the area of transplantation it seems to be also an important possible role of various trophic factors. Nerve growth factor (NGF) and Brain-derived neurotrophic factor (BDNF) in higher amount are produced by embryonic graft and may act not only in the place of transplantation (ie. in cerebellar area) but also in other brain regions. Our recent results suggest positive effect of cerebellar graft on the hippocampal long-term potentiation (LTP) compared with sham-operated controls [17].

In this preliminary study a possible influence of repetitive forced motor training in transplanted animals on their hippocampal LTP compared with controls was evaluated.

## Materials and methods

Experiments were done in Lurcher mutant mice (LMM, heterozygotes +/Lc, strain C6CBA, both sexes). Life conditions for housing were 12/12 hour light/dark cycle, water and food were available *ad libitum*. We used adult (3 month and more) LMM

( $n=47$ , body weight 20–32 g). Animals were divided into four groups according to the type of experiment: transplanted and trained ( $n=12$ ), transplanted without motor training ( $n=12$ ), sham-operated and trained ( $n=15$ ), sham-operated without motor training ( $n=8$ ).

All manipulations and methods used were performed in full agreement with the “EU Guidelines for scientific experimentation on animals”.

### *Surgery*

Embryonal cerebellar tissue was obtained from 12–13 days old foetuses without 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% sodium chloride and 0.6% glucose. Their brainstems were isolated and cerebella were dissected and pooled in the solution (0.9% sodium chloride and 0.6% glucose). Adult Lurcher mutant mice of the C6CBA strain were used as hosts. Before the transplantation, animals were 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 tissues of the occipital area of the head were 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 embryonal cerebellum (tissue obtained from one embryo) and 10  $\mu$ l of vehiculum (aqueous solution of 0.9% sodium chloride and 0.6% glucose) were injected with a glass microcapillary into the host cerebellum. Finally, the wound was sutured in one layer. To sham-operated control animals only vehiculum was administered by the same procedure [18].

### *Motor training*

Forced motor training was performed using a rotarod with cylinder diameter 4 cm and rotation speed 4 turns per minute. The training started 10 days after the surgery (transplantation or sham-operation) and it was repeated during 6 weeks, 5 days a week and in the 7<sup>th</sup> week for two more days (so 32 days altogether). On the rotarod animals spent four times 2 minutes in one day. If an animal fell down time measurement was interrupted automatically and continued again when it was returned back on the rotarod [19].

### *Hippocampal LTP*

Hippocampal long-term potentiation was performed as an acute experiment under urethane anaesthesia (20%, 1.5 g/kg b.w., intraperitoneally). After the loss of nociceptive and corneal reflexes the animal was fixed into the stereotaxic frame. Body temperature was measured by rectal probe and a small heating pad (Fine Science Tools, USA) was used for temperature keeping at  $37^{\circ}\text{C} \pm 0,5$ . After the surgical preparation and calva cleaning, the corresponding holes were prepared using a high-speed microdrill (Fine Science Tools, USA) in coordinates for the

stimulation of perforant path: (AP –  $\lambda$ , L – 3.0, V – 2.0) and for the registration from ipsilateral hilus of dentate gyrus (AP – 2.0, L – 1.7, V – 1.9). For the recording and stimulation stainless steel electrodes were used. Grounding electrode was fixed in contralateral prefrontal area to the bone with a screw. All calculations were done according to the bregma point [20].

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

The final statistical evaluation was performed by Kruskal-Wallis test using the amplitude characteristics as a comparable parameters and post-hoc Mann-Whitney test. The obtained data did not show normal distribution (verified with Kolmogorov-Smirnov test) and thus nonparametric tests were used.

## Results

The magnitude of population spike amplitude during seven time intervals after the tetanic stimulation (HFS) was analysed. Five minutes after HFS, the amplitude increased with statistical significant differences among groups ( $p < 0.0379$ ) so that maximal values were found in the transplanted animals which still underwent the motor training (Figure 1). Animals from this group also revealed highest enhancement of amplitude (140–160 %) in comparison with others during all time of measurement (ie. one hour). The second time interval with statistically significant differences between experimental and control groups was 20 minutes after HFS

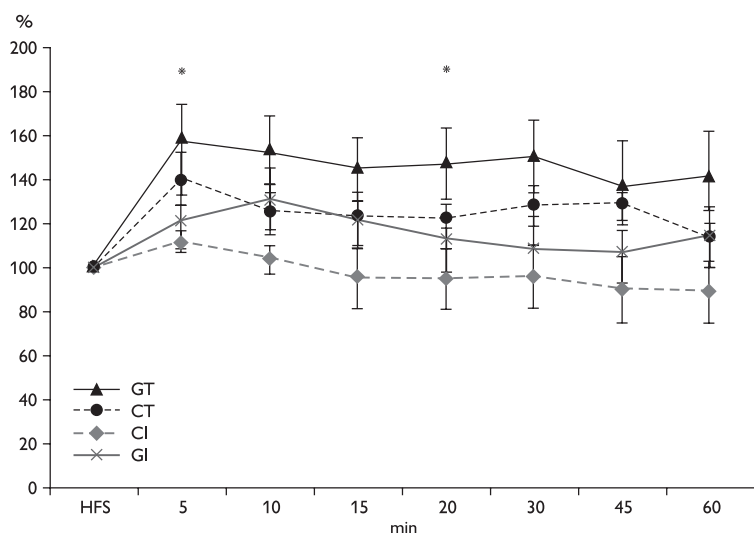


Figure 1 – LTP  
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( $p < 0.0482$ ). The worst responses after HFS were found in control (ie. sham-operated) animals without motor training. The animals of two other experimental groups (ie. transplanted without training and trained control) revealed moderate enhancement of the amplitude (in both about 120%). Detail post-hoc statistical analysis among all experimental groups is described bellow (Table 1).

## Discussion

Presented preliminary study is focussed on the possible effect of transplanted embryonic cerebellar graft on the long-term potentiation in hippocampal area with an expected dependence on the forced motor training. Recent experimental studies showed that repeated motor training improved motor abilities in adult Lurcher mutant mice [19]. Also repeated motor activity, handling and enriched environment led to significant increase of spatial learning ability which is undoubtedly hippocampal-dependent [21]. But it seems to be a relevant idea that mechanisms of spatial navigation are more complex and they involve also nonspatial components as motor control or stress factors [22]. Results of this study clearly showed that the “best” effect, ie. the highest amplitude enhancement was in the group of transplanted animals which were (a few days after surgery) trained 32 days on the rotarod. So that the combination of both influences – embryonic cerebellar graft transplantation and long-term forced motor training – had the maximal effect. On the other hand, control animals (ie. sham-operated) and without motor training revealed worse responses after the tetanic stimulation during all experimental procedures. LTP production ability in Lurcher mutant mice of this group is practically blocked and it confirms our previous findings [23, 24].

## Conclusions

Lurcher mutant mice appear as an excellent model to study the development of progressive neurodegenerative process and possibilities of influencing it [25]. Transplantation of the embryonic graft into the adult cerebellum led to positive effect on the LTP magnitude in hippocampal region and it was further improved with forced motor training. Because recent study from another hippocampal region (CA1) showed that inhibitory avoidance training induced LTP [26] we can speculate that motor learning paradigm (performed during motor training) may cause similar improvement of LTP. However, details about possible influencing between cerebellar and hippocampal areas remain still unclear and the mechanisms need further investigation.

**Table 1**

	CI vs. CT	CI vs. CT	CI vs. GI	CT vs. GT	CT vs. GI	GT vs. GI
5 min	<b>0.01693</b>	0.082589	0.969229	0.510072	<b>0.045437</b>	0.053099
20 min	<b>0.030594</b>	0.058738	0.907869	0.231909	0.097111	0.056748

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