

# Neurochemical effect of neuromuscular electrical stimulation in brain after stroke: A microdialysis study using rats with focal cerebral ischemia – A pilot study

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## Abstract

*Neuromuscular electrical stimulation (NMES) is commonly used for producing repetitive active muscular contraction, which is believed that one of the outcomes is to promote plastic reorganisation after stroke. Previous microdialysis studies showed that motor activity triggered the increase in the levels of several amino acids/ neurotransmitters such as glutamate. Massive release of glutamate during cerebral ischemia is the main pathway leading to neuronal death. The objective of this study is to investigate the effect of NMES on the levels of neurotransmitters released by the hippocampal neurons after ischemic damage. The levels were monitored by microdialysis for two weeks. Basal percentage of glutamate was found to be increased after NMES intervention and then dropped gradually. This trend persisted throughout a week (Day 1 to Day 7 after stroke), whereas the levels remained steady in control group. The increase in glutamate may lead to additional excitotoxic damage in hippocampus following the intervention. Taurine level was increased after NMES on Day 1, but this pattern did not persist afterwards. Its basal concentration kept increasing throughout 2 weeks while glutamate basal concentration exhibited an opposite trend. Taurine may act as an inhibitor to the excitotoxicity by excessive glutamate.*

electrical stimulation (NMES), which generates repetitive active muscle contraction [1], has been used as one of the post-stroke rehabilitation strategies. Liberson *et al* pioneered to use electrical stimulation to improve the gait of hemiplegic people. It assists a weak or paralyzed movement. Advanced techniques for brain imaging revealed the altered post-stroke activation patterns of specific brain structures, which may be an implication of plastic reorganization [2].

Bland *et al*'s study showed the increased level of glutamate, taurine and aspartate in hippocampus triggered by motor activity in rats. It was suggested that these neurotransmitters may play a role in recovery of motor function after brain injury [3]. Following cerebral ischemia, the excessive amounts of glutamate might activate a delayed excitotoxic neuronal injury. This excitotoxic cellular damage leads to a persistent impairment of protein synthesis in hippocampal neurons [4]. Thus, it is still unclear that whether NMES would exacerbate the brain damage or promote recovery following cerebral ischemia. This study aimed at investigating the effect of NMES on the levels of neurotransmitters (glutamate and taurine) released by the hippocampal neurons after ischemic damage. This might provide more basic understandings on the motor recovery mechanisms by neuromuscular electrical stimulation after stroke.

## 1. INTRODUCTION

Stroke is a leading cause of serious, long-term disabilities, which include loss of motor, sensory and cognitive functions. Approximately forty percent of stroke sufferers experience moderate to severe impairments who requiring special care. An effective rehabilitation strategy promotes early motor recovery and thus allows the people after stroke to reintegrate into the society as soon as possible. Neuromuscular

## 2. METHODS

The protocols for the animal experiment were performed according to the Procedures for the Care of Laboratory Animals and Codes of Ethics of the Hong Kong Polytechnic University. Eight Sprague-Dawley rats, weighted 280-300g, were used in this study. They were randomly assigned to two groups: Control group and Electrical stimulation (ES) group. They were allowed to receive water and

food *ad libitum*. Animals were anaesthetized with chloral hydrate (0.4mg/kg, i.p.). Two Teflon-coated stainless steel wires were passed subcutaneously from the incision on the left hindlimb to the exposed skull. Electrodes were made by stripping insulation off the end of the wires and looping them around the belly of tibialis muscle. The rats were then placed on stereotaxic frame. A guide cannula was implanted through a bore hole in the right hippocampus, AP: 5.8; ML: 5.0; DV: 3.0 (mm) as described by Paxinos & Watson (1996), relative to bregma. The cannula was fixed with screws and dental cement, together with the wires. One week after the implant surgery, stroke was induced in all rats using intraluminal suture technique as described [5]. Briefly, the right middle cerebral artery (MCA) was occluded by introducing a 4-0 monofilament nylon suture with rounded tip from the carotid bifurcation into the internal carotid artery until a mild resistance was felt. Reperfusion was established after 40-min occlusion, by gentle withdrawal of the suture.

Animals from ES group (n=4) were stimulated for two consecutive weeks (twice a day, 30 minutes/section, 5 days/week). They were stimulated with biphasic stimulation. Frequency of the stimulation was 20Hz and the pulse width was set to 200µsec. The stimulation was delivered by a clinical stimulator. Current was determined by increasing the current of the stimulator gradually until contraction of TA was observed, which was enough to produce a dorsiflexion of ankle joint. The rats with implanted electrodes were allowed to move freely on the treadmill while electrical stimulation was applied on the left TA muscle (see Figure 1).

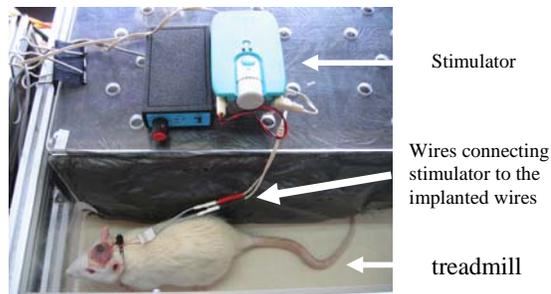


Figure 1. Rat with implanted cannula and electrodes ran on the treadmill while neuromuscular electrical stimulation was applying by the clinical stimulator.

In vivo samplings using microdialysis were carried out in all rats 2 days before inducing stroke, day 1, 2, 4, 7 and 14 after stroke. The microdialysis probe (BAS MD-2204) was

inserted through the cannula. The probes were connected to a microinfusion pump (BAS Inc.) perfused with artificial cerebrospinal fluid at a constant flow rate of  $2\mu\text{L min}^{-1}$ . The microdialysis samples were collected every 15 minutes in vials. The total collection time was about 5.5 hours. Glutamate and taurine were assayed by High Performance Liquid Chromatography (HPLC) with fluorescence detection, using the precolumn derivatisation method with ortho-phthaldialdehyde (OPA) and an automatic system from Shimadzu Instruments for HPLC (Kyoto, Japan). The extracellular amino acid levels in the dialysates are expressed in  $\mu\text{M 15min}^{-1}$ , and are not corrected for relative recovery across the dialysis membrane.

### 3. RESULTS

Data are given as means  $\pm$  SEM. Significant level was not analysed due to small sample size. Figure 2 shows the level of hippocampal glutamate (in basal percentage) changed with time and day. Glutamate was increased after ES intervention and then gradually dropped in the course of time (min) in both Day 1 and 7. The levels of glutamate in control group kept steady with time (min) in both days.

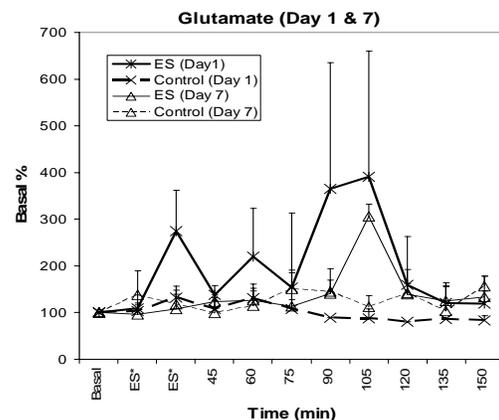


Figure 2. Effect of 30min of NMES on hippocampal glutamate levels in ES (solid lines) and Control (broken lines) animals on Day 1 (cross) and Day 7 (triangle). Data are expressed as means  $\pm$  SEM. \*Rats in control group received no intervention during ES duration.

As shown in Figure 3, the basal % level of taurine was increased after ES intervention and dropped back to basal level on Day 1, but no increase on Day 7. Similar to the glutamate levels, hippocampal taurine in control group remained at steady levels with time on both Day 1 and Day 7. According to Figure 4, basal concentration of glutamate in both groups was

decreasing, whereas taurine exhibited an increasing trend throughout the 2 weeks.

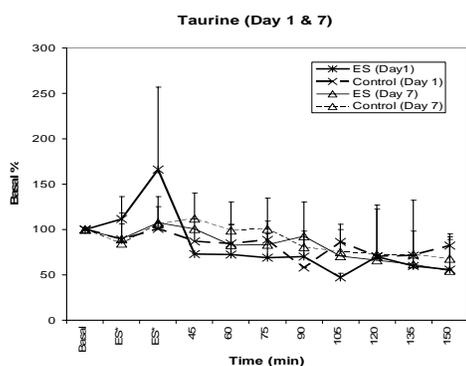


Figure 3. Effect of 30min of NMES on hippocampal Taurine levels in ES (solid lines) and Control (broken lines) animals on Day 1 (cross) and Day 7 (triangle). Data are expressed as means  $\pm$  SEM. \*Rats in control group received no intervention during ES duration

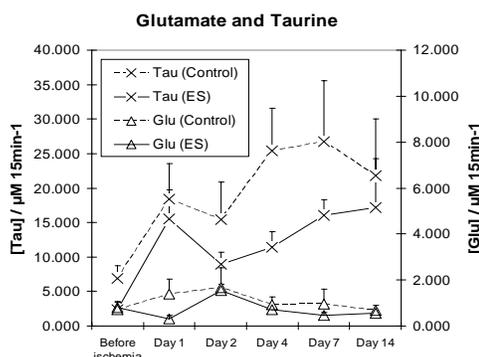


Figure 4. Basal concentration of Glutamate and Taurine in hippocampus of rats in control and ES groups before stroke and at different days after stroke. Data are presented as means  $\pm$  SEM.

#### 4. DISCUSSION AND CONCLUSIONS

In Risedal *et al's* study, cortical infarct volume was found to be larger in the early training group compared with the control group. It was suggested that in the presence of excitatory and toxic substances from ischemic tissue, an additional release of glutamate induced by motor activity may worsen the injury in the early postischemic stage [6]. In our study, there is a trend of increase in glutamate levels in ES group after NMES intervention throughout a week. It may imply additional excitotoxic damage triggered by the intervention after stroke for one week.

Some studies showed that taurine may regulate glutamate level and thus attenuate neuronal injury during ischemia [7]. Although taurine was found to be increased only on Day 1 in ES group after stroke, its basal concentration kept

increasing throughout 2 weeks (Figure 4). While taurine was increasing, glutamate level exhibited an opposite trend. Probably, there are some interactions between the actions of glutamate and taurine. Taurine may inhibit the excitotoxicity caused by excessive release of glutamate.

Further evidences such as indexes measuring the extent of brain damage (infarct volume, deficit scores, etc.) are necessary to explain the actual effect of glutamate induced by NMES on the hippocampal neuronal death. In clinical studies, functional improvement was observed in people using NMES in chronic stage of stroke, while this study showed early NMES intervention (started one day after stroke) may induce different effects. Future study will be focused on investigating whether NMES intervention prescribed at different stages of stroke would result in different neurochemical effects on the ischemic brain.

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