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Impulse magnetic stimulation facilitates synaptic regeneration in rats following sciatic nerve injury☆

Sergey A. Zhivolupov, Miroslav M. Odinak, Nariman A. Rashidov, Ludmila S. Onischenko, Igor N. Samartsev, Anton A. Jurin

Department of Neurology, Military-Medical Academy, Saint-Petersburg, 194044, Lesnoy prospect 2, Russian Federation

Abstract

The current studies describing magnetic stimulation for treatment of nervous system diseases mainly focus on transcranial magnetic stimulation and rarely focus on spinal cord magnetic stimulation. Spinal cord magnetic stimulation has been confirmed to promote neural plasticity after injuries of spinal cord, brain and peripheral nerve. To evaluate the effects of impulse magnetic stimulation of the spinal cord on peripheral nerve regneration, we compressed a 3 mm segment located in the middle third of the hip using a sterilized artery forceps to induce ischemia. Then, all animals underwent impulse magnetic stimulation of the lumbar portion of spinal crod and spinal nerve roots daily for 1 month. Electron microscopy results showed that in and below the injuryed segment, the inflammation and demyelination of neural tissue were alleviated, apoptotic cells were reduced, and injured Schwann cells and myelin fibers were repaired. These findings suggest that high-frequency impulse magnetic stimulation of spinal cord and corresponding spinal nerve roots promotes synaptic regeneration following sciatic nerve injury.

Key Words

impulse magnetic stimulation; experimental neuropathy; sciatic nerve lesion; neuroplasticity; neural regeneration

Abbreviations

IMS, impulse magnetic stimulation; MF, myelinated fibers; SC, Schwann cell

Sergey A. Zhivolupov☆, Ph.D., Professor, Department of Neurology, Military-Medical Academy, Saint-Petersburg, 194044, Lesnoy prospect 2, Russian Federation

Corresponding author: Igor N. Samartsev, M.D., Assistant professor, Department of Neurology, Military-Medical Academy, Saint-Petersburg, 194044, Lesnoy prospect 2, Russian Federation
alpinaigor@mail.ru

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INTRODUCTION

Despite all efforts to improve functional outcome following nerve injuries, the clinical results are often unpredictable and disappointing. Nowadays, increasing attention has been paid to the pathophysiological changes following nerve injury (axon sprouting, Wallerian degeneration) and the ways by which these processes can be modulated. One of the most promising techniques is magnetic stimulation. The therapeutic effectiveness of magnetic stimulation is accounted for its modulating influence on the level of neural excitability. Moreover, the revealed changes spread outside the site of stimulations affecting other functionally homologous nervous structures. Despite the broad application of non-medical methods, particularly impulse magnetic stimulation (IMS), in treatment of cerebrovascular diseases, migraine, Parkinson's disease, multiple sclerosis, there are still an insufficient amount of researches assessing the influence of IMS treatment on regenerative potential of nervous system after peripheral nerve injury[1]. It is noteworthy that significantly more attention has been paid to the transcranial magnetic stimulation, however, results regarding spinal cord stimulation are not so widely reported. The recent data show that IMS of

spinal cord can modify neuroplastic changes in different structures of nervous system, including not only gray matter of spinal cord itself but also in brain and peripheral nerves[2-3]. Repetitive magnetic stimulation of spinal cord is believed to produce positive effects on lymphangiogenesis in rat lower extremities[4]. Furthermore, a short-term (5 minutes) high-frequency magnetic stimulation of lumbar-sacral section of spinal cord in rats provides a long-term (30–40 minutes) antinociceptive effect in limbs. The latter is realized due to the activation of supraspinal opioidergic antinociceptive system[5].

The objective of this study was to study the influence of high-frequency IMS of spinal cord and corresponding spinal roots on compensatory and regenerative processes during the experimental sciatic nerve injury in rats.

RESULTS

Histological changes of nerve and muscle tissues following sciatic nerve lesion

Electron microscopy results showed that at 1 month after sciatic nerve injury, the signs of inflammation (swelling of the epineurium) in rat epineuriums were observed. Myelinated fibers (MF) inside the nerves were arranged in a lax manner and contained lots of collagen fibers. MF had signs of demyelination characterized by moderate myelin swelling and appearance of fibers with “foamy” myelin. Axons of MF had normal electronic density (Figure 1a). The structure of Schmidt-Lanterman clefts in nodes of Ranvier was fuzzy. Besides, there were Schwann cells (SCs) with morphofunctional signs of macrophages phagocyting myelin in the nerve trunk stroma (Figure 1b). SC contacting with MF and non-myelinated fibers had nuclei of atypical structure appropriate to apoptosis (Figure 1c). Along with abnormal SC, we could observe cells possessing nuclei with normal sarcoplasm density and uniformly distributed chromatin. The capillary lumen was often open whereas the cytoplasm of endotheliocytess showed swelling, the signal of inflammation. The distinctive neurohistological changes in structure of nerve fibers were not only on the level of compression but also much lower. Particularly, at 1 month after crash injury, the terminal part of nerve fibers in presynaptic zone was destructed and the space between postsynaptic folds was filled by collagen fibers. In addition, mitochondria were hardly observed in postsynaptic area of musculus gastrocnemius (Figure 2).

Histological changes of nerve and muscle tissues in sciatic nerve injury rats treated with IMS

In 1 month after the beginning of IMS treatment, the epineurium had a typical structure, whereas SC were poorly differentiated and had incomplete set of organelles. The epineurium was transparent and MFs were settled porously; the number of collagen fibers surrounding them was reduced. The MFs had moderately swollen myelin shell and axons with typical structure. The nodes of Ranvier were a bit modified (increased transparency of Schmidt-Lanterman clefts). Sections with exfoliated lamellas were observed in some MFs. The SCs had nuclei with normal sarcoplasm density, uniformly distributed chromatin and large compact nucleoli. The cytoplasm of SCs contained a large number of polysomes and tubules of granular endoplasmic reticulum representing intracellular reparation (Figure 3).

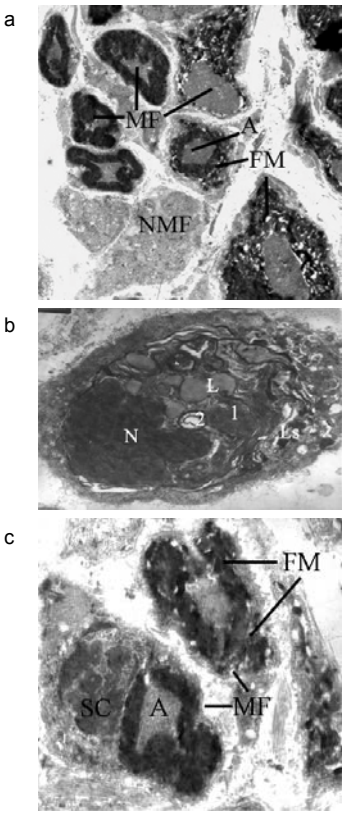

Figure 1 Ultramicroscopic observation of rat sciatic nerve stained according to the methods of Van Gizon and Mallory at 1 month after compression without treatment (electron transmission microscopy; × 5 000 in a, b; × 12 500 in c).

(a) Demyelination of fibers and appearance of foamy myelin. MF: Myelin fiber; FM: “foamy” myelin; A: axon; NMF: non-myelin fiber.

(b) Schwann cells with morphofunctional signs of macrophage. N: Nucleus; 1: myelin; 2: myelin fibers; L: lipids; Ls: lysosomes.

(c) Apoptosis of SC. SC: Schwann cells; MF: myelin fibers; FM: “foamy” myelin; A: axon.

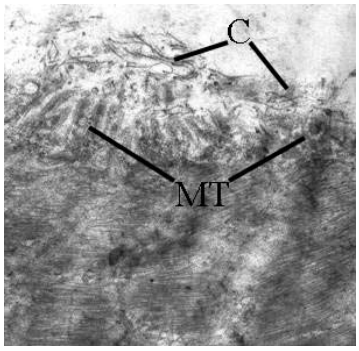

Figure 2 Ultramicroscopic observation of rat neuromuscular synapse stained according to the methods of Van Gizon and Mallory at 1 month after compression without treatment (electron transmission microscopy; × 16 000).

Pathologically changed neuromuscular synapse with nerve destruction and folds of synaptic zone filled with collagen fiber (C). Single mitochondrion (MT) in postsynaptic (muscle) zone.

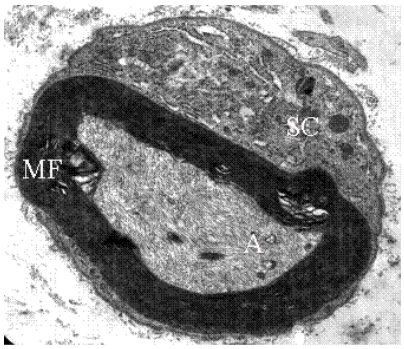

Figure 3 Ultramicroscopic observation of sciatic nerve stained according to the methods of Van Gizon and Mallory at 1 month after sciatic nerve injury and impulse magnetic stimulation treatment (electron transmission microscopy; × 16 000). SC: Schwann cell; MF: myelin fiber; A: axon.

The axons of non-myelinated nerve fibers had typical structure. The capillary lumen was mostly open; the structure of endotheliocyte nucleus and the cytoplasm were predominantly intact though cytoplasm contained fewer organelles than usual. The swelling of endotheliocytes and pericytes, indicating inflammation, was observed in some capillaries. Hypertrophic Z-discs in musculus gastrocnemius appeared along with recovery of cross-striations. Complete restoration of neuromuscular synapses reflected muscle reinnervation (Figure 4).

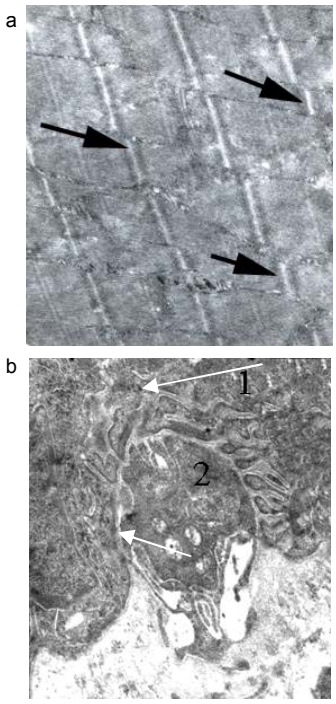

Figure 4 Ultramicroscopic observation in muscle tissue stained according to the methods of Van Gizon and Mallory at 1 month after sciatic nerve injury and impulse magnetic stimulation treatment (electron transmission microscopy; × 16 000 in a and × 25 000 in b).

(a) Recovery of cross-striation in myocytes. Hypertrophic Z-discs (arrows).

(b) Neuromuscular synapse with completely recovered structure. 1: Postsynaptic zone; 2: presynaptic zone.

DISCUSSION

Axotomy induces response from motor and sensory nerve cells that could result in both neuronal survival and regeneration or neuronal death[6]. Such neuronal death is believed to be an apoptotic response comprising pathways and mediators that are activated in various neuropathological conditions[7]. Following axotomy, neuronal apoptosis might be initiated by alterations in electrical activity, neurotoxic products of inflammation and loss of target derived neurotrophic support[8]. Furthermore, it was estimated that the loss of target-derived neurotrophic factors is the principal determinant of the extent of neuronal death after axotomy[9]. Mitochondria play a central role in this process. It has been suggested that the interaction of pro-apoptotic mediator Bax and pro-survival Bcl-2 at the mitochondrial level determines the fate of cells by governing mitochondrial outer-membrane permeability[10].

The functional goal of regeneration process is to

reinnervate the target organs and to restitute their function[9]. A peripheral nerve lesion activates a complex molecular response in the neuron. The distinct biochemical changes develop within several hours after axotomy, increasing synthesis of growth associated proteins and components of the membrane. The SC support survival and regeneration by increased synthesis of surface cell adhesion molecules. Besides, lemmocytes provide a basement membrane with extracellular matrix proteins laminin and fibronectin and produce various growth factors[6, 11]. After injury, SC in the distal stump proliferate and convert their function from myelination of electrically active axons to growth support for regenerating nerve fibers[9]. Optimal expression of growth-supportive molecules by SC is associated with macrophages infiltrating the distal stump of injured nerve[12].

The morphological study results of our experimental neuropathy revealed considerable improvement in compensatory and regenerative processes (neuroplasicity) during the application of IMS, including reduced inflammation, demyelination and apoptosing cells, and intensified SC and MF reparation. Bannaga *et al* [13] obtained similar results.

The presence of “foamy”-like non-myelinated sections in sciatic nerve of control group rats in 1 month following compression coincides with the description of similar changes received during the research of bovine spongiform encephalopathy[14]. Such changes in myelin fibers as exfoliation of lamellas and myelinophagia were also observed in optic nerves in experimental Creutzfeld-Jacob disease, Gerstman-Shtrossel disease and scarpie[15]. Similar data were also obtained in the studies of hereditary demyelinating neuropathies[16], experimental allergic encephalomyelitis[17] and diabetic neuropathy[18]. Hence, such markers of demyelination as thickening of lamellas and exfoliation are widely spread myelin pathology that could be observed in different diseases, while “foamy” myelin was described only during spongiform encephalopathy and peripheral nervous system injury. The therapeutic effectiveness of IMS is probably related to its positive influence on the functional activity of neuronal ensembles that define the regenerative potential of nervous system.

Thus, the IMS of spinal cord formations is an innovative tendency in treatment of posttraumatic neuropathies because it accelerated regeneration of the injured nerves by activating multilevel neuroplastic changes.

MATERIALS AND METHODS

Design

A controlled, observational animal experiment.

Time and setting

This study was performed at the Department of Neurology, Military Medical Academy, Saint-Petersburg, Russian Federation between May 2009 and April 2010.

Materials

Twenty white hooded rats of the Lister strain, aged 90 ± 18.5 days, weighing 230–425 g, consisting 12 males and 8 females, were included in this study. The animal care and experimental procedures were performed in accordance with the standards established by the NIH Guide for Care and Use of Laboratory Animals (National Institutes of Health Publications No. 86–23, revised 1985) and the European Communities Council Directive (86/609/EEC).

Methods

Establishment of sciatic nerve injury model

Before the beginning of experimental procedures, the rats were injected intramuscularly with fluanisone (10 mg/mL; Janssen Pharmaceuticals, Tilburg, Netherlands) and fentanyl (0.2 mg/mL; Janssen Pharmaceuticals); 1 mL/100 g body weight. The right sciatic nerve was exposed by splitting the muscles of hip. In exposed sciatic nerve, a 3 mm segment located in the middle third of the hip was toughly compressed for 30 seconds using a sterilized artery forceps and applied perpendicular to the nerve, after which the wound was stitched up in layers.

IMS treatment

At 3 days after surgical procedure, all rats (20 animals) were equally and randomly divided into a control group (without any therapy) and an experimental group in which the animals underwent IMS of the lumbar portion of spinal cord and spinal nerve roots (intensity 1 T; frequency 3 Hz; duration 3 minutes) daily during 1 month. The IMS was performed using a Cadwell MES-10, high-speed magnetic stimulator with a water-cooled figure-of-8 magnetic coil, which consists of two circular loops with a diameter of 5 cm each (Cadwell Laboratories, Inc., Kennewick, WA, USA). The coil was supported by an adjustable steel frame, and it was adjusted so that the center of the coil was in contact with the lumbar region of the experimental rats.

Histopathogical observation

In order to research the neuromorphology of the natural and modulated neuroplastic changes induced by experimental neuropathy, we conducted the electronic microscopy study of rat sciatic nerve (on the compression level and 1 cm below) and corresponding muscle fibers of musculus gastrocnemius at 1 month after the IMS sessions. The materials for the electronic

microscopy were prepared according to the standard procedures[19]. The histological sections of the sciatic nerve were stained by Van-Gizon and Mallory methods[20]. The ultrathin sections of the nerve (up to 300–500 nm) were contrasted and studied using an LEO-910 electron transmission microscope (Carl Zeiss Inc., Thornwood, NY, USA).

Author contributions: All authors contributed extensively to the work presented in this paper. Sergey A. Zhivolupov and Miroslav M. Odinak developed the concept and designed the experiment. Nariman A. Rashidov and Anton A. Jurin performed experiments. Ludmila S. Onischenko performed microscopy research. Igor N. Samartsev wrote the manuscript with the help of Sergey A. Zhivolupov and Ludmila S. Onischenko and with advice of Miroslav M. Odinak. All authors discussed the results and implications and commented on the manuscript at all stages.

Conflicts of interest: None declared.

Ethical approval: All experimental procedures were approved by the Ethics Committee of the Military Medical Academy, Saint-Petersburg, Russia (protocol No. 82, 10.06.2009).

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