Effects of swimming on functional recovery and brain-derived neurotrophic factor (BDNF) mRNA expression after sciatic crushed nerve injury in rats

Lee, Myoung-Hwa, P.T, Ph.D. · Byun, Yong-Hyun, Ph.D.
Research Institute of Sports Science, Korea University
Yoon, Bum-Chul, P.T, Ph.D.
Department of Physical Therapy, College of Health Science, Korea University
Kim, Chang-Ju, M.D.*
Department of Physiology, College of Medicine, Kyung Hee University

교신저자: 김창주, 130-701, 서울 동대문구 회기동 1번지 경희대학교 의과대학 생리학교실
Tel: +82-2-961-0282, Fax: +82-2-964-2195, E-mail: changju@khu.ac.kr

<Abstract>

말초신경은 외상이나 질병 등 여러 가지 원인으로 손상되기 쉬우며, 손상의 정도가 심하거나 치료가 지연되는 경우에는 심각한 기능 소실을 초래할 수 있다. 본 연구에서는 수영이 말초신경손상후 운동기능의 회복과 뇌유인성 신경영양인자 (brain-derived neurotrophic factor, BDNF) mRNA의 발현에 미치는 효과를 알아보기 위하여, 흰쥐 좌골신경에 압박 손상을 가하고 수영을 적용한 후 보행궤적분석 (walking track analysis)과 역전사연쇄반응 (reverse transcription-polymerase chain reaction, RT-PCR)을 실시하였다. 그 결과, 좌골신경 압박손상된 쥐는 특징적인 보행패턴을 나타내어 좌골신경기능지수 (sciatic function index, SFI)가 현저히 낮아졌으며, BDNF mRNA의 발현이 증가하였다. 좌골신경 압박 손상후 수영을 한 쥐에서는 SFI가 현저히 향상되었으며, BDNF mRNA의 발현은 억제되었다. 이러한 결과는 말초신경손상후 수영이 BDNF mRNA의 발현을 조절함으로써 기능 회복을 촉진시키는 효과적인 치료방법이 될 수 있음을 제안하고 있다.

INTRODUCTION

Peripheral nerve injury sometimes results in devastating clinical problem. Following peripheral nerve injury, the distal stump of injured axons undergoes Wallerian degeneration with breakdown of myelin sheath, recruitment of inflammatory cells from the circulation, and over-production of growth factors (Meyer et al., 1992; Funakoshi et al., 1993). Regeneration of new axons begins earlier, within 2 days after injury, but proceeds slowly (Fu and Gordon, 1997).

In many studies, the effects of various forms of therapeutic exercise on peripheral nerve regeneration and functional recovery have been investigated. Treadmill and wheel running in animals following tibial or sciatic nerve lesion are known to increase muscle mass and isometric muscle strength and energy supply to muscle (Crockett et al., 1975; Irintchev et al., 1990). After sciatic crushed nerve injury, swimming for 3 days increased the number and diameter of axons (Gutmann and Jakoubek, 1963). Recently, it was also reported that voluntary activity accelerates locomotor recovery and motor nerve conduction velocity after sciatic crushed nerve injury in rats (van Meeteren et al., 1997), and that myelin debris was removed and newly synthesized myelin fibers were observed following 4 weeks exercise in rats (Sarikcioglu and Oguz, 2001).

On the other hand, overloading by swimming and treadmill running after sciatic nerve injury was reported to have deleterious effects on parameters of nerve and muscle functions (Gutmann and Jakoubek, 1963 Herbison et al., 1974). Van Meeteren et al. (1998) reported that swimming does not affect functional sensorimotor recovery after sciatic nerve injury and that treadmill running retards functional recovery. Controversy whether exercise exerts beneficial or deleterious effect on peripheral nerve regeneration following sciatic crushed nerve injury continues to date.

Characteristic gait changes occur after unilateral sciatic nerve injury in rats. Gradual disappearance of these changes reflects nerve regeneration and functional recovery (Bervar, 2000). The current and standard method for measuring functional recovery after sciatic nerve injury in rats is the sciatic function index (SFI) established in 1982 (De Medinaceli et al., 1982), and subsequently modified in 1989 (Bain et al., 1989). SFI formula is based on the characteristic walking patterns following sciatic nerve injury in rats, and the recovery rate can be determined by this gait analysis.

Neurotrophins are a family of proteins essential for the development, maintenance, and functioning of the vertebrate nervous system (Huang and Reichardt, 2001). Of these, brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that promotes the survival of specific neurons in the central and peripheral nervous system during development (Sinder, 1994; Terenghi, 1999). In adulthood, BDNF is known as an important neuromodulator of synaptic plasticity (Pezet et al., 2002). The levels of BDNF mRNA and protein were altered following peripheral nerve injury. It was reported that BDNF mRNA was highly expressed on 3 days after sciatic nerve injury and this upregulation of BDNF mRNA expression continued during several weeks (Funakoshi et

al., 1993; Meyer et al., 1992).

In the present study, the effects of swimming on the functional recovery and BDNF mRNA expression following sciatic crushed nerve injury in rats were investigated. Functional recovery was analyzed using a walking track analysis, which can be quantified with the sciatic function index (SFI), and BDNF mRNA expression was assessed using reverse transcription-polymerase chain reaction (RT-PCR).

METHODS

Animals and Surgery

Male Sprague-Dawley rats weighing 200 ± 10 g (6 weeks of age) were used. The experimental procedures were performed in accordance with the animal care guidelines of National Institute of Health (NIH) and the Korean Academy of Medical Sciences. The animals were housed at a controlled temperature (20 ± 2℃) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lights on from 07:00 to 19:00 h), with food and water made available *ad libitum*. The rats were randomly divided into three groups (n = 8 in each group): the sham-operation group, the sciatic nerve injury group, and the sciatic nerve injury and swimming group.

Surgical procedure

To induce crush injury on the sciatic nerve in rats, a surgical procedure based on previously described method developed in 1986 was performed (De Koning et al., 1986). In brief, the right sciatic nerve was exposed through splitting incision on the gluteal muscle under pentobarbital anesthesia (50 mg/kg, i.p.; Sigma Chemical Co., St. Louis, MO, USA). The sciatic nerve was carefully exposed and crushed for 30 sec using a surgical clip between the sciatic notch and the point of trifurcation. Subsequently, the surgical wound was sutured and recovered. In the sham-operation rats, the sciatic nerve was exposed but crushing pressure on the nerve was not applied.

Swimming

After 72 h from the operation, rats of the swimming groups were made to swim for 5 min in the plastic tanks of 50 cm in height and 30 cm in diameter filled with water at 32℃ to a depth of 35 cm.

Walking track analysis

Functional recovery rate after sciatic nerve injury was analyzed using a walking track assessment, which can be quantified with SFI. Examination of the walking patterns was performed seven times at one day intervals through the course of the experiment as a previously described method (Bain et al., 1989). Footprints were recorded in a wooden walking alley (8.2 × 42 cm) with a darkened goal box at the end. The floor of the alley was covered with white paper. The anatomical landmarks on the hind feet of the rats

were smeared with finger paint. The rat was allowed to walk down the track, leaving its footprints on the paper.

From the footprints, the following parameters were calculated: distance from the heel to the top of the third toe (Print Length; PL), distance between the first and the fifth toe (Toe Spread; TS), and distance from the second to the fourth toe (Intermediary Toe Spread; IT). These parameters were taken both from the intact left (non-operated) foot (NPL, NTS, and NIT) and from the injured right (experimental) foot (EPL, ETS, and EIT). SFI values were obtained using following equation (Fig. 1):

$$SFI = -38.3\left(\frac{EPL - NPL}{NPL}\right) + 109.5\left(\frac{ETS - NTS}{NTS}\right) + 13.3\left(\frac{EIT - NIT}{NIT}\right) - 8.8$$

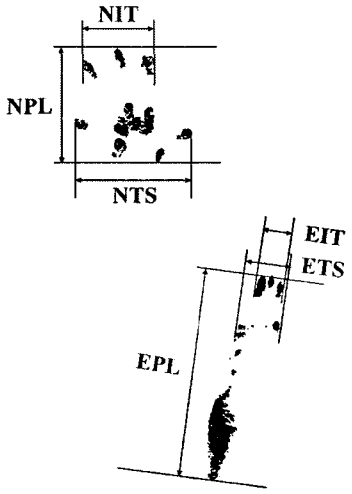

Fig. 1. Walking track analysis. After sciatic crushed nerve injury in rats, paired parameters of the print length (PL), toe spread (TS), and intermediary toe spread (IT) was taken, and these were incorporated into Bain's formula (1989). *E*, experimental side; *N*, normal side; *EPL*, experimental print length; *NPL*, normal print length; *ETS*, experimental toe spread; *NTS*, normal toe spread; *EIT*, experimental intermediary toe spread; *NIT*, normal intermediary toe spread; *SFI*, sciatic functional index

Interpolating identical values of PL, TS, and IT from the right and the left hind feet are close to zero in normal rats. A value of -100 indicates complete impairment of walking ability.

RNA isolation and RT-PCR

On the 14th days after commencement of experiment, animals were weighing and overdosed with Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France). In order

to perform RT-PCR, 5 mm of injured nerve stumps were removed and trimmed off. The samples were minced and chopped onto ice using 0.1% diethyl pyrocarbonate (DEPC) water-treated tissue scissor. After homogenization into RNAzol^{TM}B (Tel-TEST, Friendswood, TX, USA), RNA was isolated according to the manufacturer's manual. The amount of RNA was quantified according to absorbance at 260 nm (RNA/DNA calculator; Pharmacia, Uppsala, Sweden).

Single-strand cDNA was synthesized using a reaction mixture containing 2 μg of RNA template, 1 $\mu \ell$ of random primer (Promega, Madison, WI, USA) and sterile H_2O_2 for adjustment of the final volume to 10 $\mu \ell$; the template was then denaturated at 65℃ for 10 min and placed at room temperature for 5 min. One $\mu \ell$ of AMV reverse transcriptase (Promega), 5 $\mu \ell$ of 10 mM dNTP (Promega), 1 $\mu \ell$ of RNasin (Ribonuclease inhibitor; Promega), and 5 $\mu \ell$ of 10 x AMV RT buffer (Promega) were added to the mixture, and the final volume was brought up to 40 $\mu \ell$ with DEPC water. The reaction mixture was then incubated at 42℃ for 2 h.

PCR amplification was performed in a reaction volume of 40 $\mu \ell$ containing 1 $\mu \ell$ of the appropriate cDNA, 1 $\mu \ell$ of each set of primers at a concentration of 10 pM, 4 $\mu \ell$ of 10 x RT buffer, 1 $\mu \ell$ of 2.5 mM dNTP, and 2 units of *Taq* DNA polymerase (TaKaRa, Shiga, Japan). For rat *BDNF*, the primer sequences were 5′-GAGCGTGTGTGACAGTATTAG-3′(a 21-mer sense oligonucleotide starting at position 2543) and 5′-GTAGTTCGGCATTGCGAGTTC-3′ (a 21-mer anti-sense oligonucleotide starting at position 2741). For *GAPDH*, the internal control used in the study, the primer sequences were 5′-CTGCCACTCAGAAGACTGTGG-3′ (a 21-mer sense oligonucleotide starting at position 52) and 5′-CTTGATGTCATCATACTTGGC-3′ (a 21-mer anti-sense oligonucleotide starting at position 332). The expected sizes of the PCR products were 199 bp (for BDNF) and 281 bp (for GAPDH).

For *BDNF*, the PCR procedure was carried out using a GeneAmp 9600 PCR system (Perkin Elmer, Norwalk, CT, USA) under the following conditions: initial denaturation at 94℃ for 5 min, followed by 40 amplification cycles, each consisting of denaturation at 94℃ for 30 sec, annealing at 56℃ for 45 sec, and extension at 72℃ for 45 sec, with an additional extension step at the end of the procedure at 72℃ for 5 min. For *GAPDH*, the PCR procedure was carried out under identical conditions except that 30 amplification cycles were executed.

The final amount of RT-PCR product for each of the mRNA species was calculated densitometrically using Molecular Analyst^{TM}version 1.4.1 (Bio-Rad, Hercules, CA, USA). All of the BDNF values were corrected according to the GAPDH values.

Data analyses

Data were expressed as a mean ± standard error mean (S.E.M). For comparisons between groups, one-way ANOVA and Duncan's post-hoc test were performed with $p < 0.05$ as an indication of statistical significance

RESULTS

Effect of swimming on the SFI following sciatic crushed nerve injury

The mean SFI in each group was calculated on the 2nd, 4th, 6th, 8th, 10th, 12th, and 14th days after sciatic crushed nerve injury. The SFI in the sham-operation group was -9.35 ± 4.73 on the 2nd days, -10.09 ± 5.41 on the 4th days, -4.70 ± 3.79 on the 6th days, -9.99 ± 3.90 on the 8th days, -12.71 ± 2.59 on the 10th days, -6.10 ± 3.00 on the 12th days, and -9.34 ± 1.90 on the 14th days at the commencement of the experiment.

The SFI in the sciatic nerve injury group was -86.83 ± 3.33 on the 2nd days, -96.54 ± 4.69 on the 4th days, -91.89 ± 3.43on the 6th days, -100.75 ± 3.75 on the 8th days, -90.20 ± 2.54 on the 10th days, -76.47 ± 3.65 on the 12th days, and -70.83 ± 3.41 on the 14th days at the commencement of the experiment.

The SFI in the sciatic nerve injury and swimming group was -91.96 ± 3.91 on the 2nd days, -90.04 ± 4.48 on the 4th days, -82.93 ± 3.43 on the 6th days, -79.02 ± 4.63 on the 8th days, -75.04 ± 5.30 on the 10th days, -73.62 ± 2.84 on the 12th days, and -45.21 ± 12.36 on the 14th days at the commencement of the experiment (Fig. 2).

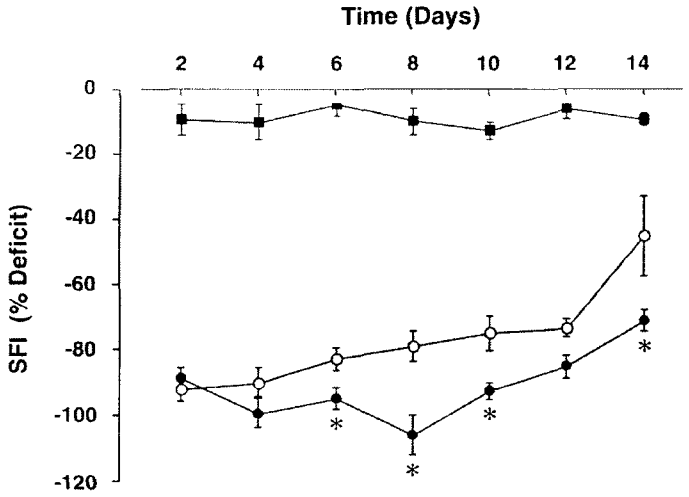

Fig. 2. Effect of swimming on the sciatic functional index (SFI) following sciatic crushed nerve injury. Values are represented as mean ± S.E.M. *difference between sciatic nerve injury group and sciatic nerve injury and swimming group, $p < 0.05$. (■) Sham-operation group, (●) sciatic nerve injury group, and (○) sciatic nerve injury and swimming group.

In the present results, the SFI of the sham-operation group continued near zero level during the experiment. At the beginning, the SFI of the both sciatic nerve injury group

and sciatic nerve injury and swimming group dropped near to -100. In the sciatic nerve injury group, SFI value was continued at the low level until 8th days after injury and then slowly increased. In the sciatic nerve injury and swimming group, enhanced SFI value was observed from the 6th day and rapidly increased through out the experiment. These results indicate that swimming promotes functional locomotor recovery following sciatic crushed nerve injury.

Effect of swimming on BDNF mRNA expression following sciatic crushed nerve injury

The level of BDNF mRNA expression in each group was measured by RT-PCR on the 14th days after starting of the experiment. The level of BDNF mRNA in the sham-operation group was set at 1.00. In the sciatic nerve injury group, the relative expression level of BDNF mRNA was markedly increased to 1.86 ± 0.16. In the sciatic nerve injury and swimming group, the relative expression level of BDNF mRNA was 1.09 ± 0.15, coming back to level of the sham-operation group, (Fig. 3).

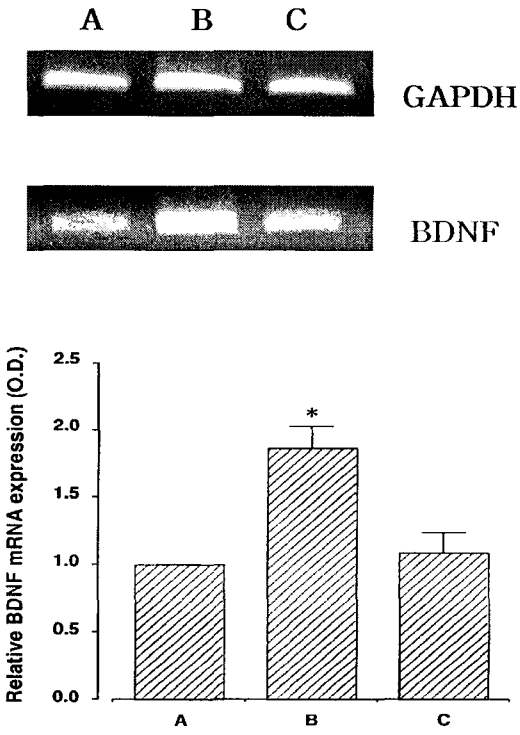

Fig. 3. Effect of swimming on the level of brain-derived neurotrophic factor (BDNF) mRNA expression. Upper: Photographs of the BDNF mRNA expression in each group. Lower: Mean BDNF mRNA level in each group. Values are represented as mean ± S.E.M. * represents $p < 0.05$ compared to the sham-operation group. # represents $p < 0.05$ compared to the sciatic nerve injury group. (A) Sham-operation group, (B) sciatic nerve injury group, and (C) sciatic nerve injury and swimming group.

In the present results, BDNF mRNA expression was enhanced by sciatic crushed nerve injury and swimming significantly suppressed the sciatic nerve injury-induced increment of BDNF mRNA expression.

DISCUSSION

Exercise is a basic therapeutic procedure in the management of neuromuscular disorders. In particular, swimming has often been recommended to patients with neuromuscular diseases. In the present study, right sciatic crushed nerve injury resulted in characteristic changes of footprint patterns showing decrease in the SFI value and swimming accelerated functional recovery from the locomotor deficit after sciatic crushed nerve injury.

The present study also demonstrated that BDNF mRNA expression was increased by sciatic crushed nerve injury. These results are consistent with previous studies that significant upregulation of neurotropic factors was observed in the Wallerian degenerated nerves (Meyer et al., 1992; Funakoshi et al., 1993). In many studies, neurotrophic factors including BDNF supplied endogenously or exogenously at the site of injured lesion have been reported to reduce motor neuron death and enhance peripheral nerve regeneration (Novikov et al., 1997; Zhang et al., 2000). Under physiological conditions BDNF synthesis is highest in the CNS, but is highly produced in the nonneuronal cells of the damaged nerve after peripheral nerve injury (Meyer et al., 1992; Zhang et al., 2000).

However, controversy showing that the infusion of a neurotrophic factor delays the onset of regeneration and suppresses regenerative responses of peripheral nerve injury in rats has been reported (Gold, 1997; Mohiuddin et al., 1999). Furthermore, Deng et al. (2000) showed that animals received BDNF antiserum could move freely without obvious reduction in locomotor activities. Therefore, it is still unclear whether excessive expression of neurotrophic factors including BDNF promotes or delays functional recovery from nerve injury.

In the present study, swimming suppressed sciatic nerve injury-induced enhancement of BDNF mRNA expression. Damage to peripheral nerves induces pain, sometimes results in peripheral neuropathic pain. It is generally accepted that injured nerve recruits immune cells such as mast cells and macrophages and that immune cells release allergic mediators, which sensitize nociceptors and induce inflammation (Theodosiou et al., 1999; Clatworthy et al., 1995). Recent studies reported that BDNF might play an important role in the induction of neuropathic pain after peripheral nerve injury (Pezet et al., 2002; Cho et al., 1997; Kim et al., 2001). Theodosiou et al. (1999) reported that systemic injection of BDNF antibodies reduced the hyperalgesia induced by the partial sciatic nerve dissection.

In addition, the nerve damage frequently results in the formation of neuroma at the site of injury. Neuroma induces severe pain and acts as a source of major disability

(Mass et al., 1984; Kryger et al., 2001). Neurotrophins including BDNF at the site of nerve injury may play an important role in the formation of neuroma. Kryger et al. (2001) showed that inhibition of NGF prevented formation of traumatic neuroma in rats. Based on the previous studies, it is possible that pain induced by over-expression of neurotropic factors including BDNF after peripheral nerve injury may lead to motor dysfunction and that inhibitory effect of swimming on BDNF expression could relieve pain, resulting in increased motor performance.

CONCLUSION

The present study suggests that over-expression of BDNF mRNA expression by sciatic nerve injury may restrain locomotor functional recovery and that swimming promotes functional recovery rate via suppression on BDNF mRNA expression. Based upon the present results, swimming aids recovery following peripheral nerve injury.

REFERENCES

Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg 1989; 83: 129-138.

Bervar M. Video analysis of standing-an alternative footprint analysis to assess functional loss following injury to the rat sciatic nerve. J Neurosci Methods 2000; 102: 109-116.

Cho HJ, Kim SY, Park MJ, et al. Expression of mRNA for brain-derived neurotrophic factor in the dorsal root ganglion following peripheral inflammation. Brain Res 1997; 749: 358-362.

Clatworthy AL, Illich PA, Castro GA, Walters ET. Role of peri-axonal inflammation in the development of thermal hyperalgesia and guarding behavior in a rat model of neuropathic pain. Neurosci Lett 1995; 184: 5-8.

Crockett JL, Edgerton VR. Exercise and restricted activity effects on reinnervated and cross-innervated skeletal muscles. J Neurol Sci 1975; 25: 1-9.

De Koning P, Brakkee JH, Gispen WH. Methods for producing a reproducible crush in the sciatic and tibial nerve of the rat and rapid and precise testing of return of sensory function. Beneficial effects of melanocortins. J Neurol Sci 1986; 74: 237-246.

De Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol 1982; 77: 634-643.

Deng YS, Zhong JH, Zhou XF. Effects of endogenous neurotrophins on sympathetic sprouting in the dorsal root ganglia and allodynia following spinal nerve injury. Exp Neurol 2000; 164: 344-350.

Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol. 1997; 14: 67-116.

Funakoshi H, Frisen J, Barbany G, et al. Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. J Cell Biol 1993; 123: 455-465.

Gold BG. Axonal regeneration of sensory nerves is delayed by continuous intrathecal infusion of nerve growth factor. Neuroscience 1997; 76: 1153-1158.

Gutmann E, Jakoubek B. Effect of increased motor activity on regeneration of the peripheral nerve in young rats. Physiol Bohemoslov 1963; 12: 463-468.

Herbison GJ, Jaweed MM, Ditunno JF. Effect of swimming on reinnervation of rat skeletal muscle. J Neurol Neurosurg Psychiatry. 1974; 37: 1247-1251.

Huang EJ, Reichardt LF. Neurophins: roles in neuronal development and function. Annu Rev Neurosci 2001; 24: 677-736.

Irintchev A, Draguhn A, Wernig A. Reinnervation and recovery of mouse soleus muscle after long-term denervation. Neuroscience 1990; 39: 231-243.

Kim DS, Lee SJ, Cho HJ. Differential usage of multiple brain-derived neurotrophic factor promoter in rat dorsal root ganglia following peripheral nerve injuries and

inflammation. Brain Res Mol Brain Res 2001; 92: 167-171.

Kryger GS, Kryger Z, Zhang F, et al. Nerve growth factor inhibition prevents traumatic neuroma formation in the rat. J Hand Surg 2001; 26: 635-644.

Mass DP, Ciano MC, Tortosa R, et al. Treatment of painful hand neuromas by their transfer into bone. Plast Reconstr Surg 1984; 74: 182-185.

Meyer M, Matsuoka I, Wetmore C, et al. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. J Cell Biol 1992; 119: 45-54.

Mohiuddin L, Delcroix JD, Fernyhough P, Tomlinson DR. Focally administered nerve growth factor suppresses molecular regenerative responses of axotomized peripheral afferents in rats. Neuroscience 1999; 91: 265-271.

Novikov L, Novikova L, Kellerth JO. Brain-derived neurotrophic factor promotes axonal regeneration and long-term survival of adult rat spinal motoneurons in vivo. Neuroscience 1997; 79: 765-774.

Pezet S, Malcangio M, McMahon SB. BDNF: a neuromodulator in nociceptive pathways? Brain Res Brain Res Rev 2002; 40: 240-249.

Sarikcioglu L, Oguz N. Exercise training and axonal regeneration after sciatic nerve injury. Int J Neurosci 2001; 109: 173-177.

Sinder WD. Functions of the neurotrophins during nervous system development: What the knockouts are teaching us. Cell 1994; 77: 627-638.

Terenghi G. Peripheral nerve regeneration and neurotrophic factors. J Anat 1999; 194: 1-14.

Theodosiou M, Rush RA, Zhou XF, et al. Hyperalgesia due to nerve damage: role of nerve growth factor. Pain 1999; 81: 245-255.

van Meeteren NL, Brakkee JH, Hamers FP, et al. Exercise training improves functional recovery and motor nerve conduction velocity after sciatic nerve crush lesion in the rat. Arch Phys Med Rehabil 1997; 78: 70-77.

van Meeteren NL, Brakkee JH, Helders PJ, Gispen WH. The effect of exercise training on functional recovery after sciatic nerve crush in the rat. J Peripher Nerv Syst. 1998; 3: 277-282.

Zhang JY, Luo XG, Xian CJ, et al. Endogenous BDNF is required for myelination and regeneration of injured sciatic nerve in rodents. Eur J Neurosci 2000; 12: 4171-4180.