

Ectopic Muscle Expression of Neurotrophic Factors Improves Recovery After Nerve Injury

Micaela Johanna Glat^{1,3} · Felix Benninger² · Yael Barhum³ · Tali Ben-Zur³ · Elena Kogan² · Israel Steiner² · David Yaffe⁴ · Daniel Offen^{1,3,5}

Received: 6 August 2015 / Accepted: 26 August 2015 / Published online: 18 September 2015
© Springer Science+Business Media New York 2015

Abstract Sciatic nerve damage is a common medical problem. The main causes include direct trauma, prolonged external nerve compression, and pressure from disk herniation. Possible complications include leg numbness and the loss of motor control. In mild cases, conservative treatment is feasible. However, following severe injury, recovery may not be possible. Neuronal regeneration, survival, and maintenance can be achieved by neurotrophic factors (NTFs). In this study, we examined the potency of combining brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1) on the recovery of motor neuron function after crush injury of the sciatic nerve. We show that combined NTF application increases the survival of motor neurons exposed to a hypoxic environment. The ectopic expression of NTFs in the injured muscle improves

the recovery of the sciatic nerve after crush injury. A significantly faster recovery of compound muscle action potential (CMAP) amplitude and conduction velocity is observed after muscle injections of viral vectors expressing a mixture of the four NTF genes. Our findings suggest a rationale for using genetic treatment with a combination of NTF-expressing vectors, as a potential therapeutic approach for severe peripheral nerve injury.

Keywords Neurotrophic factors · Compound muscle action potential (CMAP) · Conduction velocity · Nerve injury

Introduction

Neurotrophic factors (NTFs) are a family of peptides, which mediate neurons survival and regeneration in the central and peripheral nervous systems (Boyd and Gordon 2003). NTFs may be part of future treatment options for peripheral nerve injury since they have been shown to possess a therapeutic effect on axonal regeneration (Bregman et al. 1997; Shiotani et al. 1998; Terenghi 1999; Chen et al. 2010; Moimas et al. 2013). Brain-derived neurotrophic factor (BDNF) regulates development, neurogenesis, maintenance, and survival of different populations of neurons (Meyer et al. 1992; Binder and Scharfman 2004). BDNF was also shown to prevent the loss of motor units and to enhance branching and arborization of motor neurons (Fryer et al. 2000; Mousavi et al. 2002; Hu and Kalb 2003; Özdinler and Macklis 2006). Glial-derived neurotrophic factor (GDNF) is another survival factor of central and peripheral neurons and is essential for the development of the nervous system (Sánchez et al. 1996). GDNF prevents neuronal degeneration in mice and rats after axotomy, as well as precludes the apoptosis of motor neurons during development (Zhao et al. 2004). An additional key survival factor for

Micaela Johanna Glat and Felix Benninger contributed equally to this work

Electronic supplementary material The online version of this article (doi:10.1007/s12031-015-0648-9) contains supplementary material, which is available to authorized users.

✉ Daniel Offen
danioffen@gmail.com

- ¹ Sagol School of Neuroscience, Tel-Aviv University, Tel-Aviv, Israel
- ² Department of Neurology, Rabin Medical Center, Petach Tikva, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel
- ³ Felsenstein Medical Research Center, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel
- ⁴ Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel
- ⁵ The Neuroscience Laboratory, Felsenstein Medical Research Center, Rabin Medical Center, 49100 Petach Tikva, Israel

peripheral neurons is the insulin growth factor 1 (IGF-1). In the SOD1 transgenic mouse model of amyotrophic lateral sclerosis (ALS), overexpression of GDNF or IGF-1 in muscles resulted in hyper-innervation of the muscles by motor neurons (Rabinovsky et al. 1992; Özdinler and Macklis 2006). Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis that promotes the recovery in a number of models of neural trauma (Schratzberger et al. 2001; Widenfalk et al. 2003; Pan et al. 2006).

The sciatic nerve is the longest nerve in the human body extending from the lower part of the spinal cord to the buttocks and down the legs. Damage to the sciatic nerve (sciatica) is one of the most common reasons for peripheral neuropathy. This includes direct trauma, tumors, cysts, infection, lumbar herniated disk, or other insults. The symptoms are persistent leg and foot pain, numbness, and weakness possibly leading to permanent disability (Fitzimmons et al. 2014).

We hypothesized that the combined administration of several NTFs, differing in molecular pathways and cell types in which they operate, may lead to a synergistic or additive effect. Indeed, using the injured sciatic nerve model, we have shown that intramuscular injection of a mixture of myogenic cells, expressing together several NTFs, is superior to administration of single NTFs (Dadon-Nachum et al. 2012). Moreover, injections of such cells delay the onset of symptoms and improve survival in the SOD1 mouse model of ALS (Dadon-Nachum et al. 2015). In the present study, we have examined the effect of direct intramuscular administration of combinations of NTFs encoding genes, in a mouse model of sciatic nerve injury. Using nerve conduction studies (NCS), we show that administration of a mixture of vectors expressing combinations of *BDNF*, *GDNF*, *IGF-1*, and *VEGF* improved significantly the recovery of axonal function.

Materials and Methods

Exposure of Cells to Hypoxia-Reoxygenation and Cell Viability Measurements The mouse NSC-34 hybrid cell line was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin/streptomycin (PS), and 1 % L-glutamine solution at 37 °C in an atmosphere of 5 % CO₂. Cells were then plated in 96-well plates (Corning) at 15,000 cells per well in 100 µl DMEM supplemented with 100 mg/ml streptomycin, 100 U/ml penicillin, 12.5 units/ml nystatin 2 mM L-glutamine, and 10 % fetal calf serum (Biological Industries, Beit Haemek, Israel). After 24 h, various combinations of BDNF (1 ng; Peprotech: 450-02-2), GDNF (10 ng; Peprotech: 450-10-2), IGF-1 (200 ng; Peprotech: 100-11-100), or VEGF (0.15 ng; Peprotech: 100-20-2) were added to the NSC-34 cells. After 1 h incubation, the cells with the factors were exposed to hypoxia (1 % oxygen, 5 % CO₂, and

94 % N₂) for 48 h. Alamar blue 10 % (AbD Serotec, Kidlington, UK) was added to the cells for 3 h. The results were read at a wavelength of 590 nm using a fluostar device (Synergy HT, BioTeck Instruments, Inc., VT, USA). Each experiment was repeated at least three times.

Gene Cloning and Lentiviral Preparation The human *GDNF*, *VEGF*, *IGF-1*, and *BDNF* genes were amplified from pBluescript plasmids that were purchased from Harvard Institute of Proteomics, Boston, USA. Each gene and its CMV promoter were cloned into the pLenti6/R4R2/V5-DEST using the ViraPower Promoterless Lentiviral Gateway Kit (Invitrogen, Carlsbad, CA, USA). The constructs (3 µg) were co-transfected with the packaging plasmids (9 µg): pLP1, pLP2, and pLP/VSVG using Lipofectamine 2000 (Invitrogen) into the 293 T producer cell line. The lentiviral titer was determined using the Lenti-X p24 Rapid Titer Kit and the manufacturer's recommended procedure (Clontech, Mountain View, CA, USA).

Sciatic Nerve Injury Eight-week-old male C57bl mice ($n = 162$; Harlan, Jerusalem, Israel) were placed under 12-h-light/12-h-dark conditions and grown in individually ventilated cages (IVC) with free access to food and water. All experimental procedures were approved by the Tel Aviv University Committee of Animal Use for Research and Education. Every effort was made to reduce the number of mice used and to minimize their suffering. The mice were unilaterally injected into the right gastrocnemius muscle with the lenti-viruses, at a total volume of 50 µl (titers 3×10^5 – 2×10^8). Twelve days after the viral transfection, the mice were anesthetized with a mixture of ketamine-xylazine (100 mg/kg ketamine, 5 mg/kg xylazine). The right sciatic nerve was exposed, and a vessel clamp was applied for 30 s above the first branching of the nerve (Dadon-Nachum et al. 2012).

Pinch Test Mice were examined for sensory response by toe pinching the hind digits with tweezers, 3, 7, and 11 days after sciatic nerve crush. Foot withdrawal in response to the pinch test was scored as a percentage of responding digits.

Nerve Conduction Study For nerve conduction studies (NCS), we used the Nihon Kohden Neuropak M1 MEB-9200 EMG/EP System (MFI Medical Equipment, Inc., San Diego, California, USA). Mice were anesthetized with ketamine and xylazine (100 and 10 mg/kg, respectively) intraperitoneally, prior to the experiment. Electrodes were externally fixed using non-invasive clips (ground electrode at the tail, recording electrodes on the foot as well as on the gastrocnemius muscle). Stimulations were performed using a bipolar electrode placed either just before the gastrocnemius muscle or in a second recording location, exactly 10 mm proximal to the first recording location. Pulses were standardized (20 mA

for 2 ms) and only maximum responses were included. Recordings were performed in standardized settings and at a stable room temperature (20–21 °C). Latency and amplitude of compound muscle action potential (CMAP) were recorded. Nerve conduction velocity was calculated from two distant recordings (10 mm). The experimenter (F.B.) was blinded throughout the experiment.

Statistical Analysis

The results are expressed as means ± standard error (SE). Statistical analysis was performed using one-way-ANOVAs followed by Dunnett’s multiple comparison tests to compare groups. For direct comparison of two groups, unpaired Student’s *t* test was used. A significance level of *p* ≤ 0.05 was set for all statistical tests. Statistical analysis of data sets was carried out with the aid of GraphPad Prism for Windows (Graphpad Software, CA, USA).

Immunohistochemistry To analyze expression of NTFs in muscle tissue, mouse gastrocnemius muscles were removed and frozen in liquid nitrogen. Muscles were sliced at 30 μm using a cryostat (Leica CM1850, Nussloch, Germany) and mounted on glass slides. The sections were fixed with 4 % paraformaldehyde for 15 min, washed with phosphate-buffered saline (PBS), and then incubated in a blocking solution (5 % normal goat serum and 0.5 % Triton X-100 in PBS) for 1 h. After the blocking stage, sections were treated with a primary antibody and incubated overnight at 4 °C. Following incubation, the slices were washed in PBS three times for 5 min each. The sections were incubated with an Alexa-conjugated secondary antibody. The nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI; 1:1000; Sigma-Aldrich, St. Louis, MO, USA). The following primary antibodies were used: rabbit α-BDNF (1:200), rabbit α-GDNF (1:200), mouse α-IGF-1 (1:200), and rabbit α-VEGF (1:200; all purchased from Santa Cruz Biotechnology Inc.

Dallas TX, USA). Secondary antibodies were Alexa Fluor 488(1:500) and Alexa Fluor 568 (1:500; Invitrogen).

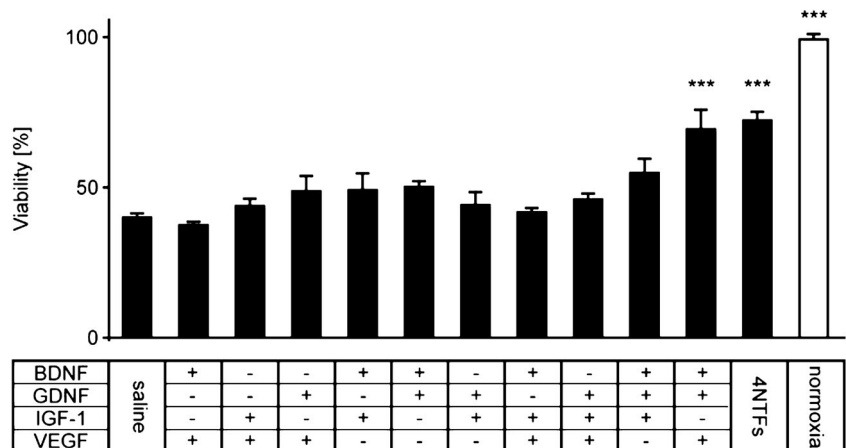
Results

Exposure to a Mixture of NTFs Alleviates the Toxic Effect of Hypoxia on Neuronal Cell Cultures Cultures of the motor neuron-like cell line NSC-34 were exposed to a hypoxic environment of 1 % oxygen for 48 h. In this hypoxic environment, only 40 % of the NSC-34 cells survived compared to cells grown in a normoxia atmosphere. Cells treated with a single NTF, 1 ng/ml of BDNF, 10 ng/ml of GDNF, 200 ng/ml of IGF-1, or 0.15 ng/ml of VEGF, did not differ in viability from the untreated cells (data not shown). When we tested various combinations of two NTFs together, no significant effect on cell survival was seen in cultures. However, NSC-34 cells treated with mixture of GDNF, VEGF, and IGF-1 or with BDNF, GDNF, and VEGF showed a significant improvement in cell survival (55 and 69 %, respectively, Fig. 1). Further improvement in cell survival was demonstrated when all four NTFs were applied, 73 vs. 40 % in untreated cells (Fig. 1). Thus, a combination of all four NTFs demonstrates marked improvement, over the other combinations.

Increased NTFs Expression in Gastrocnemius Muscles After Transfection To determine the expression of the transfected NTFs and GFP in the injected muscle, mice were sacrificed 5 weeks after viral injection. We observed GFP-labeled cells in the site of injection in the gastrocnemius (Fig. 2a, b). In muscles injected with the vectors expressing the NTFs, we observed increased levels of BDNF, GDNF, IGF-1, or VEGF, compared to untreated muscles (Fig. 2c–j).

Ectopic Expression of NTF Genes Improved Compound Muscle Action Potential (CMAP) Recovery, After Nerve Crush Injury In order to overexpress the NTFs in the muscles before the injury, we injected Lenti-viruses carrying the genes,

Fig. 1 NTF combinations partially rescue NSC-34 cells from hypoxia. NSC-34 cells were exposed to a hypoxic environment for 48 h with combinations of two, three, or four NTFs. Cell viability of NSC-34 cell was assessed by Alamar blue (***)*p* < 0.001, average ± SE



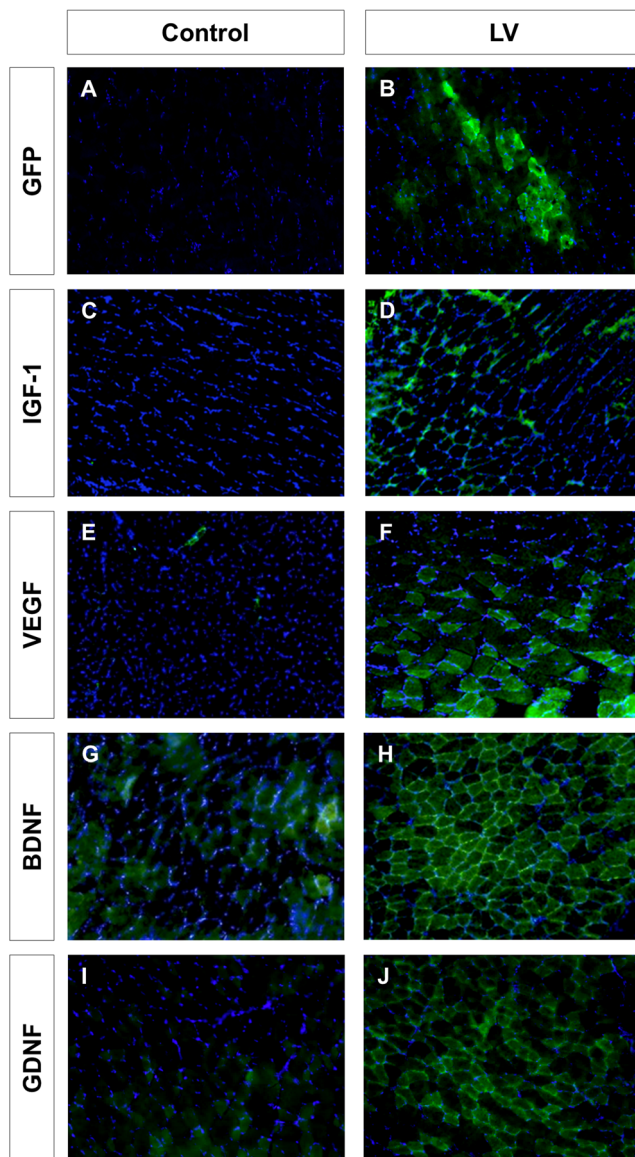


Fig. 2 Expression of NTFs in the injected gastrocnemius muscle. Mice were injected intramuscularly with saline only in the left hind limb (*left column*) or with lentiviruses carrying BDNF, GDNF, VEGF, or IGF-1 genes in the right hind limb (*right column*). GFP expression in muscle section of mice injected with saline (**a**) or LV-GFP (**b**). Muscle sections were stained for IGF-1 (**c, d**), VEGF (**e, f**), BDNF (**g, h**), and (**i, j**) GDNF. DAPI was used for nuclear staining. BDNF, GDNF, IGF-1, and VEGF staining revealed an increase in the levels of each NTF in the treated area (**d, f, h, j**), compared to muscles from uninjured and untreated mice (**c, e, g, h, i**). Scale bar 100 μ m

12 days prior to the sciatic nerve crush. BDNF, GDNF, IGF-1, or VEGF encoding genes were injected into the right gastrocnemius muscle. EMG was performed as described above and an example of the CMAP from two consecutive EMG measurements is shown before the crush injury (Fig. 3a) and 1 day after crush injury (Fig. 3b). The percentage of CMAP amplitude was normalized to pre-crush values. The level of nerve damage due to the crush injury between the groups was

comparable indicated by similar decreases in CMAP in the GFP-treated and the NTF-treated mice 1 day after the crush injury (21.1 ± 4.2 vs. 22.2 ± 1.9 , $p = 0.80$, $n = 47$). Initially, we established a dose response curve by using four different concentrations of viral vectors (BDNF [n] 1×10^6 [6], 3×10^6 [6], 12×10^6 [6], 50×10^6 [5]; GDNF [n] 1×10^6 [6], 3×10^6 [5], 12×10^6 [4], 50×10^6 [5]; IGF-1 [n] 1.25×10^7 [6], 2.5×10^7 [6], 1×10^8 [6], 2×10^8 [4]; VEGF [n] 1.25×10^7 [6], 2.5×10^7 [6], 1×10^8 [6], 2×10^8 [6]). NCS recordings, at day 11 following nerve crush, showed a dose response increased recovery of CMAP amplitude in BDNF, GDNF, and IGF-1 treated mice, compared to GFP injected animals (Fig. 3c–e; Supplement Table ST1). Mice treated with VEGF showed improvement of CMAP, compared to GFP-injected mice, but lacked a clear dose response (Fig. 3f; Supplement Table ST1).

A Mixture of all Four NTFs Improved the Recovery After Nerve Crush Injury To detect a possible synergistic effect of the NTFs on recovery of nerve damage, we injected mice with vectors expressing combinations of three NTFs or all four NTFs. The recovery effect was evaluated by CMAP amplitude. For each NTF, we used the lowest amount of lentivirus particles that showed an effect in recovering SNI (Fig. 3). CMAP recovery was followed by EMG measurements on days 1, 3, 7, and 11 after crush injury. We found that mice treated with four NTFs recovered faster than the GFP-treated mice and groups treated with various combinations of three-gene. The effect was demonstrated already on day 3 and persistent throughout the experiment (Fig. 4, Supplement Table ST2).

Conduction velocity (CV) of the sciatic nerve was an additional measurement to determine recover of the function after a crush injury. On the first day, the CV of the injured right sciatic nerve was reduced compared to the CV of the healthy left side (Fig. 5). Three days after injury, we observed in the four NTF-treated mice marked improvement, compared to the GFP-treated mice. One week after the insult, the four NTF CV values were increased significantly and were close to those of the healthy legs (Fig. 5, Supplement Table ST3).

Ectopic Muscle Expression of the Four NTF Genes Increased Sensory Fiber Recovery The sensory fiber regeneration was evaluated using the pinch test. One day after sciatic nerve crush, the reflex response was barely detectable. On days 3, 7, and 11, we observed that mice treated with the four NTFs significantly improved the reflex response compared to GFP-injected mice (Fig. 6).

Discussion

Damage to the sciatic nerve is a very common peripheral neuropathy with various causes that can lead to permanent disability (Fitzimmons et al. 2014). Mechanical pressure on

Fig. 3 NTFs improve compound muscle action potential (CMAP) after nerve crush injury. CMAP records of the gastrocnemius muscle after proximal stimulation of the sciatic nerve. The recorded CMAP is shown for two consecutive stimulations before (a) and 1 day after (b) nerve crush injury. Note the decreased amplitude (mV) after crush injury. c–f Graphic display of the extent of recovery of CMAP amplitude 11 days after crush injury, compared to pre-crush stimulations. For each NTF, the fraction of recovery is displayed according to the quantity of lentivirus injected. In each graph, the fraction of recovery after the injection of GFP expression vectors is displayed for comparison (white bar). Significant dose dependent improvement with higher amounts of NTFs is shown for c BDNF, d GDNF, and e IGF-1. As described in the text, f VEGF improved recovery significantly compared to GFP injection, but was dose independent. $p < 0.05$ is indicated by an asterisk as change from GFP injection ($n = 4$, $*p < 0.05$, average \pm SE)

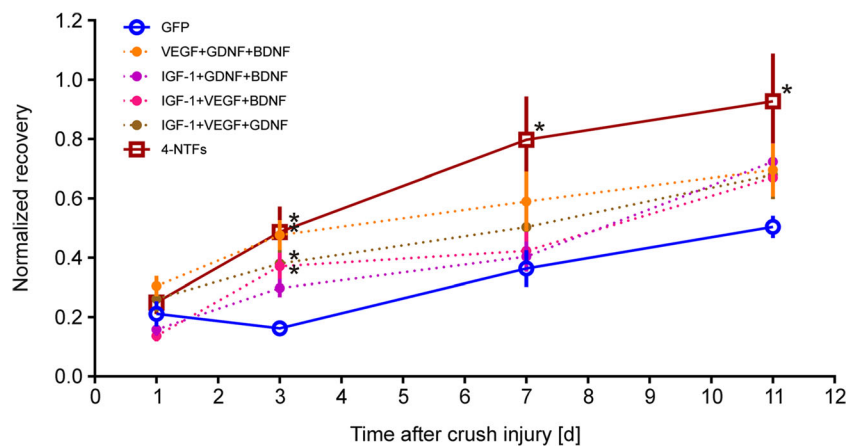
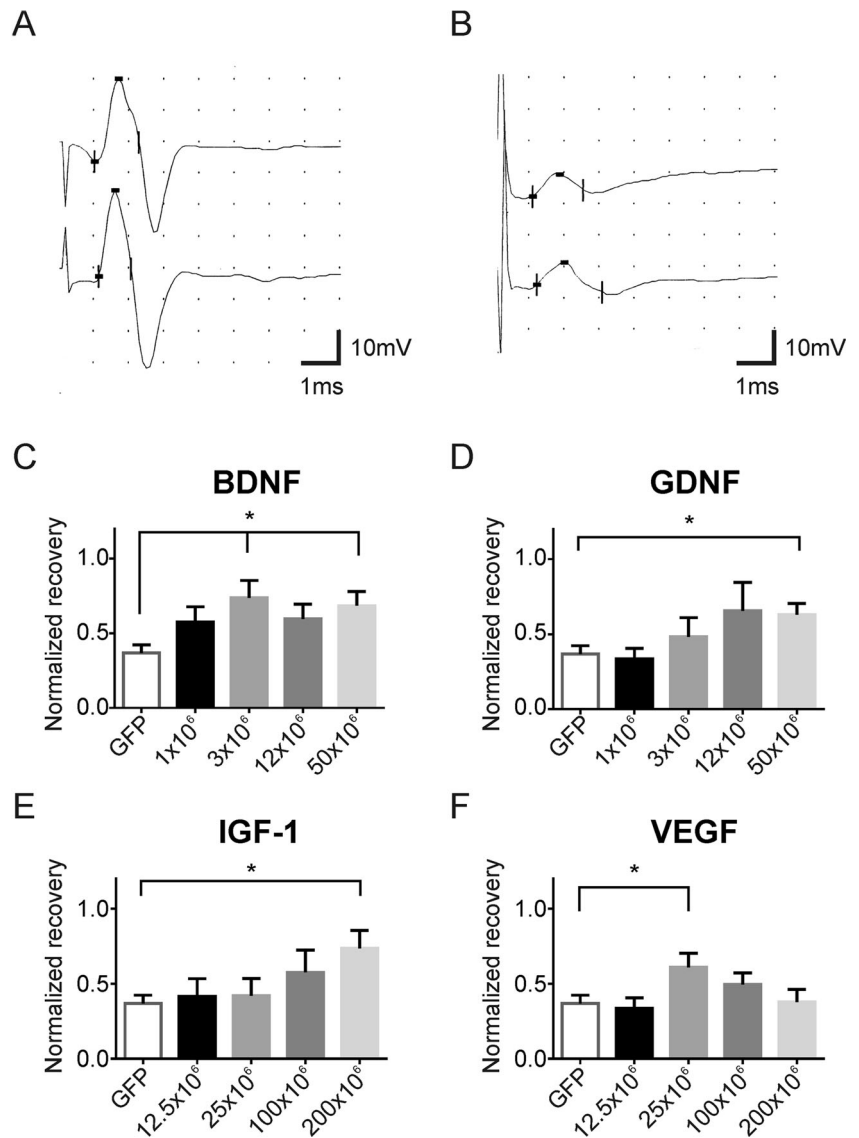
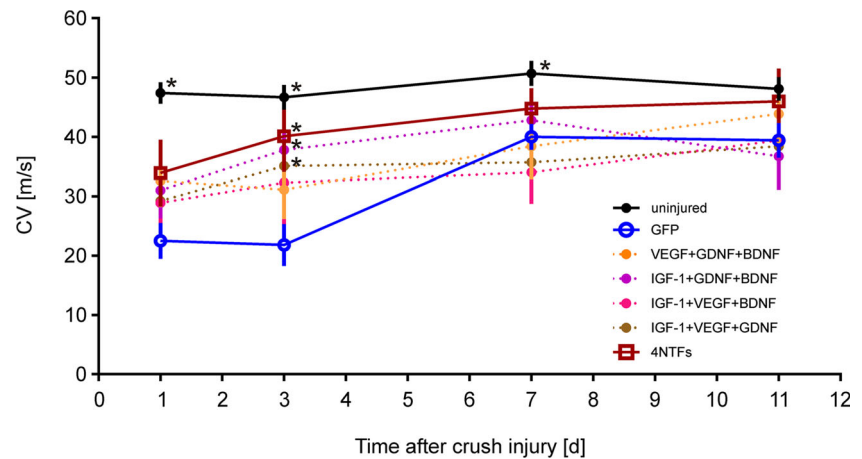


Fig. 4 Combining NTFs significantly accelerate CMAP recovery after nerve crush injury. Graphic display of the recovery of CMAP amplitude of the gastrocnemius muscle at 1, 3, 7, and 11 days after crush injury of the sciatic nerve compared to pre-crush stimulations. For each NTF-combination, the fraction of recovery is displayed. $p < 0.05$ is indicated

by an asterisk as change from GFP injection (GFP $n = 10$; VEGF + GDNF + BDNF $n = 11$; IGF-1 + GDNF + BDNF $n = 9$; IGF-1 + VEGF + BDNF $n = 8$; IGF-1 + VEGF + GDNF $n = 9$; 4-NTFs $n = 9$; $*p < 0.05$, average \pm SE)

Fig. 5 Nerve conduction velocity recovery is enhanced by exposure to a combination of all four NTFs. Graphic display of the conduction velocity (meters per second; m/s) at 1, 3, 7, and 11 days after crush injury of the sciatic nerve. The CVs of uninjured mice without crush injury ($n = 10$), GFP, four NTFs, and various NTF combinations as indicated. Asterisk indicates $p < 0.05$ a significant difference from the GFP-injected mice ($*p < 0.05$, average \pm SE)



the nerve itself deprives the axon from metabolic supply, resulting in a loss of function and permanent damage.

The present study describes the effect of ectopic muscle expression of BDNF, GDNF, IGF-1, and VEGF on the recovery of the sciatic nerve after crush injury. We show that NTFs, transferred by intramuscular lentivirus injection, accelerated the recuperation of the sciatic nerve as indicated by EMG, CMAP amplitude, and CV. The combined four NTFs significantly improved also the sensory responses as indicated by the pinch test.

The role of NTFs in peripheral nerve regeneration has been the subject of many studies. Various NTFs have shown to have therapeutic effect on injured neurons (Mohajeri et al. 1999; Acsadi et al. 2002; Pan et al. 2006; Pan et al. 2007). However, under certain circumstances NTFs may also cause adverse effects. Injection of adenoviruses carrying VEGF into the muscle of injured rabbits led to hind limb edema and the excess growth of capillaries (Vajanto et al. 2002). Kim et al. (2003) showed that prolonged BDNF exposure induced

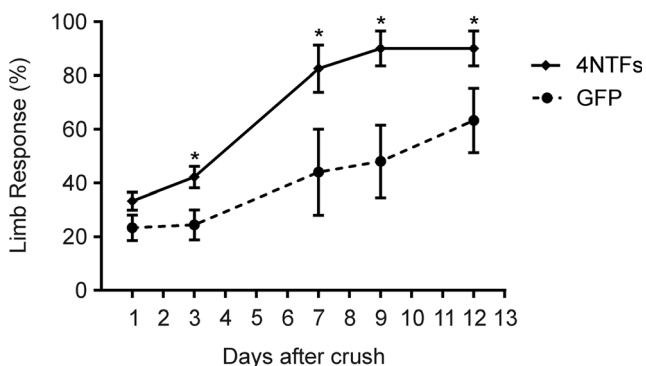


Fig. 6 Recovery of sensory responses after lesion of the sciatic nerve. Mice were lesioned at the sciatic nerve (8–9 mice per group) and were then monitored up to 12 days post lesion. At each monitoring session, the mouse's response to toe pinch was tested on each individual digit. Response was measured as percent of responding digits. One day post lesion, there was a decrease in response in both groups of mice. Mice injected with a combination of all four NTFs genes showed a significant better recovery compared to mice injected with GFP-expressing vector

oxidative neuronal necrosis in a cortical culture (Kim et al. 2003). In light of these contrasting findings, we examined the injection of increasing amounts of lentiviruses carrying each of four NTF genes to assess the minimal load of viral vectors needed to improve axonal regeneration. We found that mice treated with BDNF, GDNF, or IGF-1 showed improvement in CMAP in a dose dependent manner (Fig. 2). In contrast, in mice treated with VEGF, the improvement had a bell shape pattern. This finding is in accordance with the results indicating that expression of low levels of VEGF in myoblasts promoted growth while high expression of VEGF resulted in hemangiomas (Ozawa et al. 2004).

Several studies indicated the synergistic effect of NTFs on nerve recovery (Boyd and Gordon 2003; Chen et al. 2010; Dadon-Nachum et al. 2012; Dadon-Nachum et al. 2015). For example, in a rat model of axotomy, a combined treatment of GDNF and BDNF improved the motor neuron regeneration more than each factor alone (Boyd and Gordon 2003). Additionally, inoculation of human mesenchymal stem cells (hMSCs) modified to release GDNF and VEGF resulted in a synergistic positive effect on lifespan and on disease progression in a familial ALS rat model (Krakora et al. 2013).

In a previous study, we showed that injection of a combination of myogenic cells, overexpressing the abovementioned four NTFs, into the muscle, synergistically improved motor function in a rat model of sciatic nerve injury and in a mouse model of ALS (Dadon-Nachum et al. 2012, 2015). In the current study, we examined the effect of direct intramuscular administration of lentiviruses expressing four NTFs. We found that the NTFs are highly expressed and accelerate the recovery after sciatic nerve injury. This approach bypasses the use of cell culture infection and transplantation of in vitro genetically manipulated cells. Injection of modified cells can also induce severe immune rejection complications which might exert a negative effect. The direct application of the four NTFs by a single injection of the expression vectors is more compatible with the translational aspect. Although lentiviruses are currently used in numerous clinical studies, another

delivery system such as adeno-associated virus and novel non-integrated vectors should be tested.

In order to obtain high levels of NTFs immediately after crush injury, we injected lentiviruses carrying NTF genes 12 days prior to the nerve crush. Immediately after the crush injury, no significant differences regarding CMAP amplitude or CV were detected between the control and NTF injected mice (Figs. 3 and 4); however, we cannot exclude a protective effect. Future experiments should test the therapeutic effect of injections hours or days after the crush.

The data suggest a possible basis for clinical applications of four NTFs to enhance regeneration and recovery after nerve injury, reducing long-term immobility and disability.

References

- Acsadi G, Anguelov RA, Yang H, et al (2002) Increased survival and function of SOD1 mice after glial cell-derived neurotrophic factor gene therapy. *Hum Gene Ther* 13:1047–1059
- Binder DK, Scharfman HE (2004) Brain-derived neurotrophic factor. *Growth Factors* 22:123–131
- Boyd J, Gordon T (2003) Glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor sustain the axonal regeneration of chronically axotomized motoneurons in vivo. *Exp Neurol* 183:610–619
- Bregman BS, McAtee M, Dai HN, Kuhn PL (1997) Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat. *Exp Neurol* 148:475–494
- Chen J, Chu YF, Chen JM, Li BC (2010) Synergistic effects of NGF, CNTF and GDNF on functional recovery following sciatic nerve injury in rats. *Adv Med Sci* 55:32–42
- Dadon-Nachum M, Ben-Zur T, Srugo I, et al (2012) Therapeutic effect of myogenic cells modified to express neurotrophic factors in a rat model of sciatic nerve injury. *J Stem Cells Regen Med* 8:21–27
- Dadon-Nachum M, Ben-Yaacov K, Ben-Zur T, et al (2015) Transplanted modified muscle progenitor cells expressing a mixture of neurotrophic factors delay disease onset and enhance survival in the SOD1 mouse model of ALS. *J Mol Neurosci* 55:788–797
- Fitzimmons D, Phillips CJ, Bennett H, et al (2014) Cost effectiveness of different management strategies for sciatica. *Pain* 155:1318–1327
- Fryer HJL, Wolf DH, Knox RJ, et al (2000) Brain-derived neurotrophic factor induces excitotoxic sensitivity in cultured embryonic rat spinal motor neurons through activation of the phosphatidylinositol 3-kinase pathway. *J Neurochem* 74:582–595
- Hu P, Kalb RG (2003) BDNF heightens the sensitivity of motor neurons to excitotoxic insults through activation of TrkB. *J Neurochem* 84:1421–1430
- Kim H-J, Hwang JJ, Behrens MM, et al (2003) TrkB mediates BDNF-induced potentiation of neuronal necrosis in cortical culture. *Neurobiol Dis* 14:110–119
- Krakora D, Mulcrone P, Meyer M, et al (2013) Synergistic effects of GDNF and VEGF on lifespan and disease progression in a familial ALS rat model. *Mol Ther* 21:1602–1610
- Meyer M, Matsuoka I, Wetmore C, et al (1992) 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* 119:45–54
- Mohajeri MH, Figlewicz DA, Bohn MC (1999) Intramuscular grafts of myoblasts genetically modified to secrete glial cell line-derived neurotrophic factor prevent motoneuron loss and disease progression in a mouse model of familial amyotrophic lateral sclerosis. *Hum Gene Ther* 10:1853–1866
- Moimas S, Novati F, Ronchi G, et al (2013) Effect of vascular endothelial growth factor gene therapy on post-traumatic peripheral nerve regeneration and denervation-related muscle atrophy. *Gene Ther* 20:1014–1021
- Mousavi K, Miranda W, Parry DJ (2002) Neurotrophic factors enhance the survival of muscle fibers in EDL, but not SOL, after neonatal nerve injury. *Am J Physiol Cell Physiol* 283:C950–C959
- Ozawa Y, Hayashi K, Wakino S, et al (2004) Free radical activity depends on underlying vasoconstrictors in renal microcirculation. *Clin Exp Hypertens* 26:219–229
- Özdinler PH, Macklis JD (2006) IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. *Nat Neurosci* 9:1371–1381
- Pan H-C, Yang D-Y, Chiu Y-T, et al (2006) Enhanced regeneration in injured sciatic nerve by human amniotic mesenchymal stem cell. *J Clin Neurosci* 13:570–575
- Pan H-C, Cheng F-C, Chen C-J, et al (2007) Post-injury regeneration in rat sciatic nerve facilitated by neurotrophic factors secreted by amniotic fluid mesenchymal stem cells. *J Clin Neurosci* 14:1089–1098
- Rabinovsky ED, Smith GM, Browder DP, et al (1992) Peripheral nerve injury down-regulates CNTF expression in adult rat sciatic nerves. *J Neurosci Res* 31:188–192
- Sánchez MP, Silos-Santiago I, Frisén J, et al (1996) Renal agenesis and the absence of enteric neurons in mice lacking GDNF. *Nature* 382:70–73
- Schratzberger P, Walter DH, Rittig K, et al (2001) Reversal of experimental diabetic neuropathy by VEGF gene transfer. *J Clin Invest* 107:1083–1092
- Shiotani A, O'Malley BW, Coleman ME, et al (1998) Reinnervation of motor endplates and increased muscle fiber size after human insulin-like growth factor I gene transfer into the paralyzed larynx. *Hum Gene Ther* 9:2039–2047
- Terenghi G (1999) Peripheral nerve regeneration and neurotrophic factors. *J Anat* 194:1–14
- Vajanto I, Rissanen TT, Rutanen J, et al (2002) Evaluation of angiogenesis and side effects in ischemic rabbit hindlimbs after intramuscular injection of adenoviral vectors encoding VEGF and LacZ. *J Gene Med* 4:371–380
- Widenfalk J, Lipson A, Jubran M, et al (2003) Vascular endothelial growth factor improves functional outcome and decreases secondary degeneration in experimental spinal cord contusion injury. *Neuroscience* 120:951–960
- Zhao Z, Alam S, Oppenheim RW, et al (2004) Overexpression of glial cell line-derived neurotrophic factor in the CNS rescues motoneurons from programmed cell death and promotes their long-term survival following axotomy. *Exp Neurol* 190:356–372