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Short Term Voluntary Exercise Alters GDNF Protein Expression in Rat Spinal Cord

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Short term voluntary exercise alters GDNF protein expression in rat spinal cord

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Abstract

Glial cell line-derived neurotrophic factor (GDNF) is considered to be one of the most potent neurotrophic factors for motor neurons of the spinal cord. However, little is known about the response of GDNF in the spinal cord following exercise from healthy individuals. **PURPOSE:** Previous studies from our laboratory have shown that long term voluntary exercise (6 weeks and 6 months) has no effect on GDNF protein content in rat spinal cord. Therefore, the aim of the current study was to examine changes in GDNF protein content in the spinal cord following 2 weeks of voluntary exercise from adult and aged rats. **METHODS:** Male Sprague Dawley rats aged 6 months and 2 years were examined. The exercised animals were housed with continuous access to voluntary running wheels for 2 weeks. Age-matched sedentary control animals had no exposure to the voluntary wheels. GDNF protein content of the spinal cord was measured using an enzyme-linked immunosorbent assay (ELISA) and western blot analysis. Immunohistochemical analysis was performed to localize GDNF in nerve cells in the spinal cord. **RESULTS:** The 6-month-old animals that had undergone 2 weeks of voluntary exercise ran an average distance of 25,828.7 m whereas the 2-year-old animals ran an average distance of 1683.2 m. Total GDNF protein content in the spinal cord of 6 month old animals was significantly decreased following 2 weeks of voluntary exercise (1293.4 188.9 pg GDNF) as compared to sedentary control animals (2298.6 374.5 pg GDNF). Two weeks of voluntary exercise for the spinal cord of the 2 year old animals was approaching significant increase of total GDNF ($p = 0.08$) when compared to age-matched sedentary control animals (1797.8 370.1 pg GDNF and 792.0 222.2 pg GDNF, respectively). **CONCLUSION:** Short term voluntary exercise may increase GDNF protein content in the spinal cord of aged individuals and may act as a measure to prevent motor neuron loss commonly associated with senescence.
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Introduction

One major problem with aging individuals is the continuous loss of somatic motor neurons that innervate skeletal muscles. Increased amounts of physical activity have been shown to prevent the loss of motor neurons in aging individuals (Kanda and Hashizume, 1998).

Glial cell line-derived neurotrophic factor (GDNF) is one of the most potent trophic substances for motor neurons that innervate skeletal muscles. GDNF rescues somatic motor neurons from natural occurring cell death (Oppenheim et al., 2000), rescues motor neurons from axotomy-induced cell death (Oppenheim et al., 1995), and protects motor neurons from chronic degeneration (Corse et al., 1999). If GDNF protein expression is controlled by physical activity, then increasing levels of physical activity may reverse age-related changes of the motor neuron structure and function.

Hypothesis: Voluntary exercise will increase GDNF protein content of the spinal cord.

Aims

- **Does GDNF protein content decrease in the spinal cord in aging animals?**
- **Does short term voluntary exercise increase GDNF protein content in the spinal cord?**

Methods

Test Subjects:

23 male Sprague Dawley rats (Charles River Co.) aged 6 months (n = 12) and 2 years old (n = 11).

Sedentary (11 animals): Age-matched control animals had no running wheel access.

Exercised (12 animals): Animals were housed in individual cages with continuous access to voluntary running wheels for 2 weeks.

Determination of GDNF protein content:

Spinal cords were removed and the lumbar region was processed. The sections smashed into a powder that was homogenized in a buffered salt solution containing protease inhibitors followed by centrifugation at 23,700 x g for 30 minutes. The supernatant was collected and GDNF protein content was measured using an enzyme-linked immunosorbant assay (ELISA). GDNF values were quantified using a known standard curve and expressed as pg of total.

Statistical Analysis:

Data are represented as the mean Standard Error of the Mean (SEM). Data were analyzed using one-way ANOVA to test for differences among the independent groups. Significance is established as $p \leq 0.05$.

Immunohistochemistry:

Spinal cord sections were removed and fixed in 4% paraformaldehyde overnight at 4 C. Tissues were then washed in phosphate buffered saline (PBS) and placed in a 30% sucrose solution overnight at 4 C for cryoprotection. Tissues were stored at -80 C until ready for processing. The spinal cord was sectioned on the cryostat. Fixed tissues were bound with antibodies against GDNF, choline acetyltransferase (ChAT), and calcitonin gene related peptide (CGRP). Tissues were washed and then bound with secondary antibodies conjugated to fluorescent molecules (AlexaFluor 488, AlexaFluor 568 and AlexaFluor 647, respectively). Samples were placed on slides and viewed using a confocal microscope.

Western Blot:

Homogenized tissue supernatant was used to determine protein concentration using a BCA protein assay with bovine serum albumin as the protein standard. Samples were prepared for loading into polyacrylamide gels by adding Laemmli 2X Loading buffer and boiled for 5 minutes. A protein ladder and a positive control of a GDNF standard were added to the gel. The gel was run at two different voltages from a power source, 100 Volts though the stacking gel and 200 Volts through the loading gel. Once all the samples migrated to the bottom, the gel was transferred to a polyvinylidene difluoride (PVDF) membrane where it was transferred by a power supply of 12 volts for 1 hour. Once the transfer finished, bands from the protein ladder were marked for visualization, and the PVDF membrane was blocked non-specifically for 1 hour at 4 C under agitation. The membrane was incubated with a primary antibody against GDNF overnight at 4 C under agitation. The membrane was washed under agitation, then incubated with a HRP-conjugated secondary antibody for 1 hour at 37 C under agitation. The ECL detection kit was used to visualize the proteins and x-ray films were developed.

Results

6 weeks and 6 months of voluntary exercise does not change GDNF protein content in the Spinal Cord.

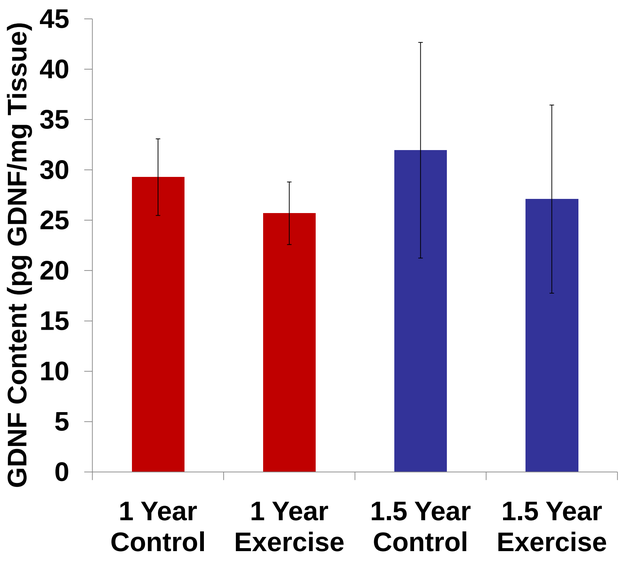

Figure1. GDNF protein content in spinal cord measured via ELISA. Spinal cord was taken from both control animals aged 1 year and exercised animals with 6 weeks of voluntary exercise, and from control animals aged 1.5 years and exercised animals with 6 months of voluntary exercise. Data represent the mean SEM ($p \leq 0.05$).

2 weeks of voluntary exercise increases GDNF protein content in the Spinal Cord of 6 month old animals.

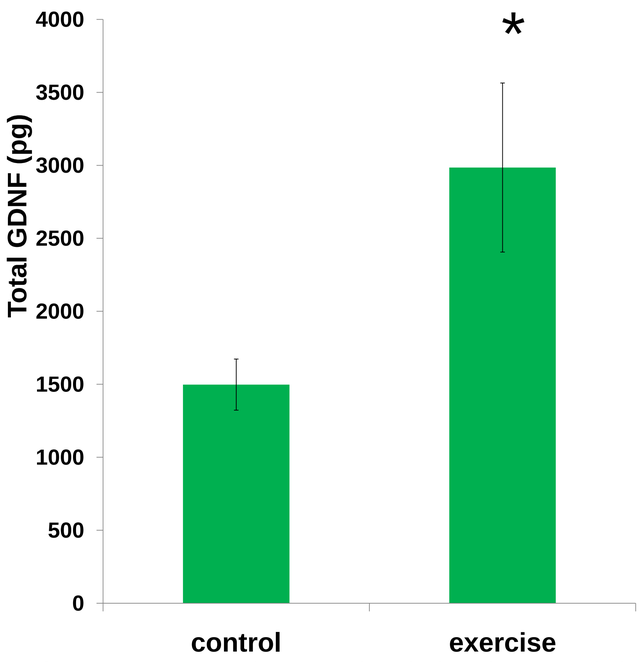

Figure 2. GDNF protein content in spinal cord measured via ELISA. Spinal cord was taken from both control animals aged 6 months and exercised animals with 2 weeks of voluntary exercise. Data represent the mean SEM ($p \leq 0.05$).

2 weeks of voluntary exercise increases GDNF protein content in the Spinal Cord of 2 year old animals.

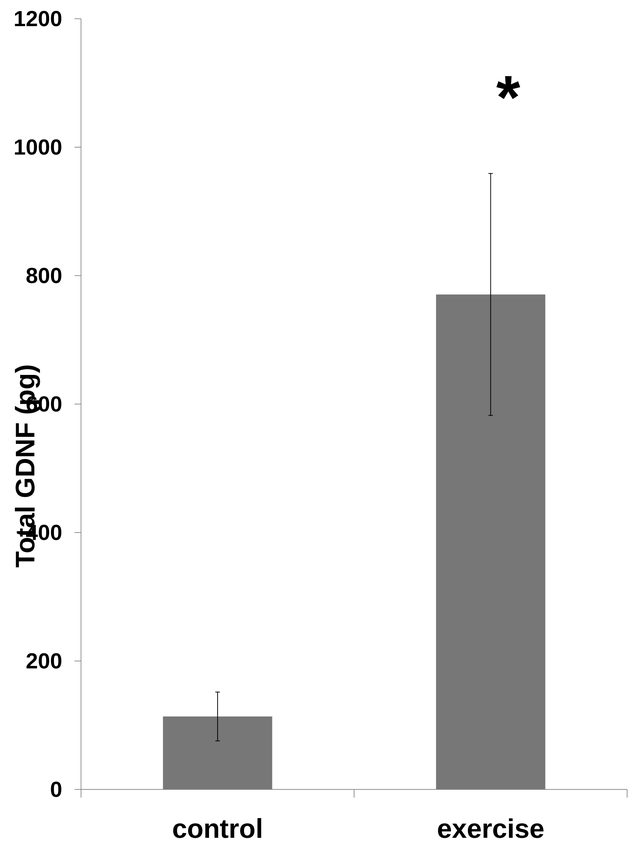

Figure 3. GDNF protein content in spinal cord measured via ELISA. Spinal cord was taken from both control animals aged 2 years and exercised animals with 2 weeks of voluntary exercise. Data represent the mean SEM ($p \leq 0.05$).

2 year old Control Spinal Cord

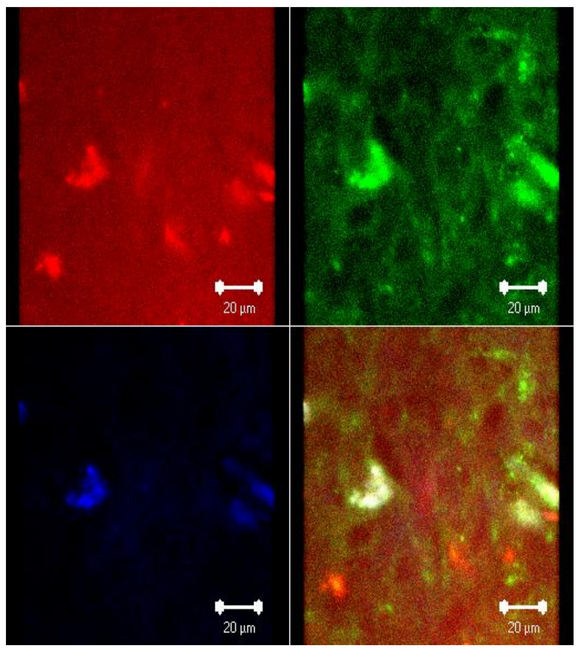

Figure 4. Lumbar segments of the spinal cord from 2-year-old control animals were fixed and bound with rabbit anti-GDNF (green), mouse anti-ChAT (red), and goat anti-CGRP (blue) and were viewed on a confocal microscope.

Exercised 2 year old Spinal Cord

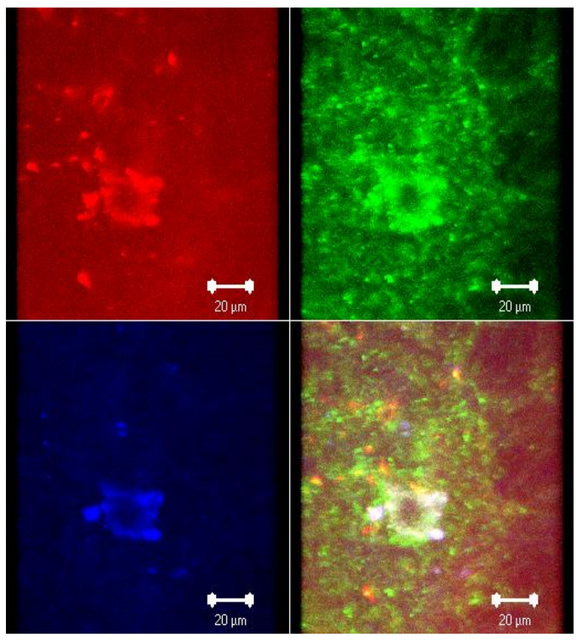

Figure 5. Lumbar segments of the spinal cord from 2-year-old animals that were exercised for 2 weeks. Sections were fixed and bound with rabbit anti-GDNF (green), mouse anti-ChAT (red), and goat anti-CGRP (blue) and were viewed on a confocal microscope.

GDNF protein size changes with exercise in the Spinal Cord.

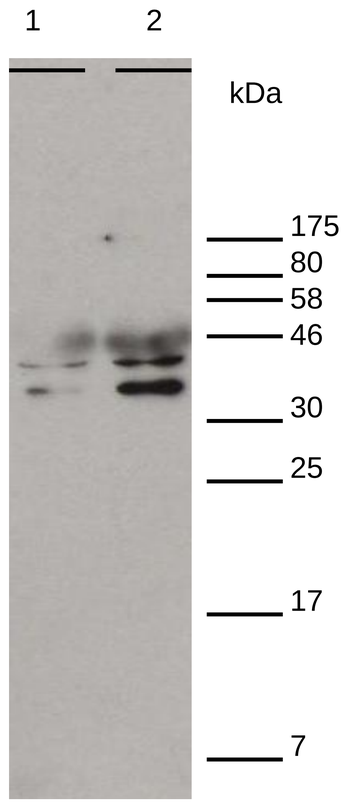

Figure 6. Western blot using antibodies specific for GDNF. Spinal cord was taken from control animals aged 6 months, and 6 month old animals with 2 weeks of exercised. Lane 1 is the 6 month old control sample. Lane 2 is the 6 month old exercised animal.

Summary

- **2 weeks of voluntary exercise increases GDNF protein content in spinal cord of 6-month-old and 2-year-old rats**
- **Exercise may increase the size of motor neuron cell bodies in spinal cord**
- **Exercise may change the isoform of GDNF protein found in the spinal cord**
- **Short term exercise may increase GDNF protein content more than long term exercise**

Conclusions

These results show that short term voluntary increases GDNF protein levels in the spinal cord of young and old animals. Immunohistochemical results suggest that motor neuron size is affected by exercise. Our western blot results suggest that expression of GDNF is higher following voluntary exercise in the spinal cord. Short term voluntary exercise may increase GDNF protein content in the spinal cord of aged individuals and may act as a measure to prevent motor neuron loss commonly associated with senescence.

Acknowledgements

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