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Confocal visualization of the effect of short-term locomotor exercise on BDNF and TrkB distribution in the lumbar spinal cord of the rat: The enhancement of BDNF in dendrites?

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Abstract. Locomotor exercise increases neurotrophin BDNF and its receptor $TrkB^{FL}$ expression in the lumbar spinal cord. Involvement of BDNF/$TrkB^{FL}$ in synaptic transmission raises the questions which intracellular compartments are involved in this upregulation and whether exercise leads to redistribution of these proteins related to the duration of exercise. We have investigated the influence of short-term (7 days) locomotor exercise (ST) on intracellular distribution of BDNF and $TrkB^{FL}$ in the rat lumbar spinal cord comparing it with the effects of long-term (28 days) exercise (LT) described earlier. Immunofluorescence (IF) of proteins was analyzed with confocal microscopy. ST exercise caused a redistribution of perikaryonal BDNF IF toward periphery resulting in an increase of dendritic signal. In contrast to an enhancement of perikaryonal BDNF staining following LT, no increase of BDNF IF in cell bodies was observed after ST. An increase of $TrkB^{FL}$ IF in oligodendrocytes was consistent with that caused by LT. The fibers of $TrkB^{FL}$ IF oligodendrocytes surrounding the largest neurons were in close apposition to neuronal membrane. We propose that ST exercise causes (1) BDNF translocation to dendrites and/or local dendritic synthesis to serve increased synaptic activity (2) sensitization of oligodendroglia to BDNF mediated responses.

Short communication

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Physical exercise has been shown to promote neurogenesis in the adult brain (van Praag et al.1999), facilitate functional recovery following brain and spinal cord injury (Barbeau and Rossignol 1987, Grealy et al. 1999) and improve memory and cognition (Fordyce and Wehner 1993, Gobbo and O'Mara 2005, Kramer and Hahn 1999, Neeper et al. 1996,Vaynman and Ying 2004).

Recent advances in understanding of the possible molecular mechanism underlying these effects of exercise revealed the role of neurotrophins in activity-dependent plasticity and suggest a central role for brain derived neurotrophic factor (BDNF) (Gobbo and O'Mara 2005, Klintsova et al. 2004, Macias et al. 2002, Neeper et al.1996, Skup et al. 2000, 2002). Our former studies have documented that long-term locomotor exercise led to an enhancement of BDNF protein and mRNA level in the spinal cord of adult rat (Macias et al. 2002, Skup et al. 2002). It was accompanied by up-regulation of full length TrkB ($TrkB^{FL}$) receptor, particularly in small cells of the ventral horn, identified as oligodendroglial cells (Skup et al. 2002). Whereas short term exercise (for 3–7 days) has been reported by the others to stimulate BDNF and TrkB gene expression in the brain and to increase BDNF protein both in the brain and in spinal cord (Gomez-Pinilla et al. 2001, Molteni et al. 2002, Neeper et al 1996), the intracellular compartments involved in upregulation and possible redistribution of these proteins in the spinal cord related to the duration of exercise were not determined. This prompted us to ask the following questions: (i) whether the observed effects depend on the duration of locomotor exercise; (ii) which intracellular compartments are involved in BDNF and TrkB upregulation due to exercise; (iii) does locomotor activity lead to redistribution of these proteins in the intracellular compartments of spinal neurons.

In this paper we show the effect of short-term (7 days) locomotor exercise on the intracellular distribution of BDNF and $TrkB^{FL}$ protein in the lumbar spinal cord of adult rats, revealed with confocal microscopy, and refer it to our former observations (Macias et al. 2002, Skup et al. 2002).

Eight adult male Wistar rats were used in the study. Animals were given free access to water and pellet food and were housed under standard humidity and temperature and 12-h light/dark cycle. Procedures involving animals and their care were in accordance with the European Committee Council Directive of 24 November 1986 (86/609/EEC) and approved by the Local Ethics Committee. The locomotor exercise was carried out for 7 days (2 rats) according to the protocol described earlier (Skup et al. 2002). Exercised rats walked on a treadmill about 1000 m daily at a speed between 20 and 25 cm/s. Control animals (2 rats) were never exercised, but were handled and rewarded in the same way as the exercised group. Four animals (2 controls and 2 long-term exercised rats) were used in an additional experiment aimed to verify cell phenotype with triple labeling. Two hours after the last training session rats were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and perfused with 0.01 M phosphate-buffered saline (PBS), pH 7.4, followed by 400–500 ml of ice-cold fixative (4% PFA). Spinal cords were removed and tissue was then cryoprotected overnight in 10% sucrose in 0.1 M PB at 4°C followed by 30% sucrose until the tissue sank. The spinal cord L3–L4 segments were frozen with precooled heptane and 16 μm glassmounted transverse sections were collected and frozen at -20°C until used. Immunocytochemical procedures were as described earlier (Skup at al. 2002). The primary antibodies used were: anti-TrkB full-length 794; sc-12 (dilution 1:400) and anti-BDNF N-20; sc-546 (1:400), purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, USA), NeuN (1:5000), purchased from Chemicon (USA). Alexa Fluor conjugates (dilution 1:100) with goat anti-rabbit and anti-mouse $F(ab)_2$ fragments (Molecular Probes, USA) were used for the fluorescence detection. Hoechst staining (bisbenzimide N. 33258, Sigma) was used to identify cell nuclei in triple-labeling experiment. Standard immunocytochemistry with DAB signal detection was carried out in parallel for comparison. The immunofluorescence was examined using a Leica TCS SP2 confocal microscope. To get insight into the intracellular distribution of the labeled proteins the 1 μm depth scans were registered and analyzed. For qualitative comparisons single scans were then superimposed to reconstruct the complete signal in the section.

Analysis of the images obtained with a confocal microscopy revealed that in control animals BDNF IF is present in neurons of the spinal gray and in fibers of gray and white matter (Fig.1a), confirming the pattern of BDNF localization characterized by us previously with bright field microscopy (Skup et al. 2002). BDNF IF was detected predominantly in large neurons

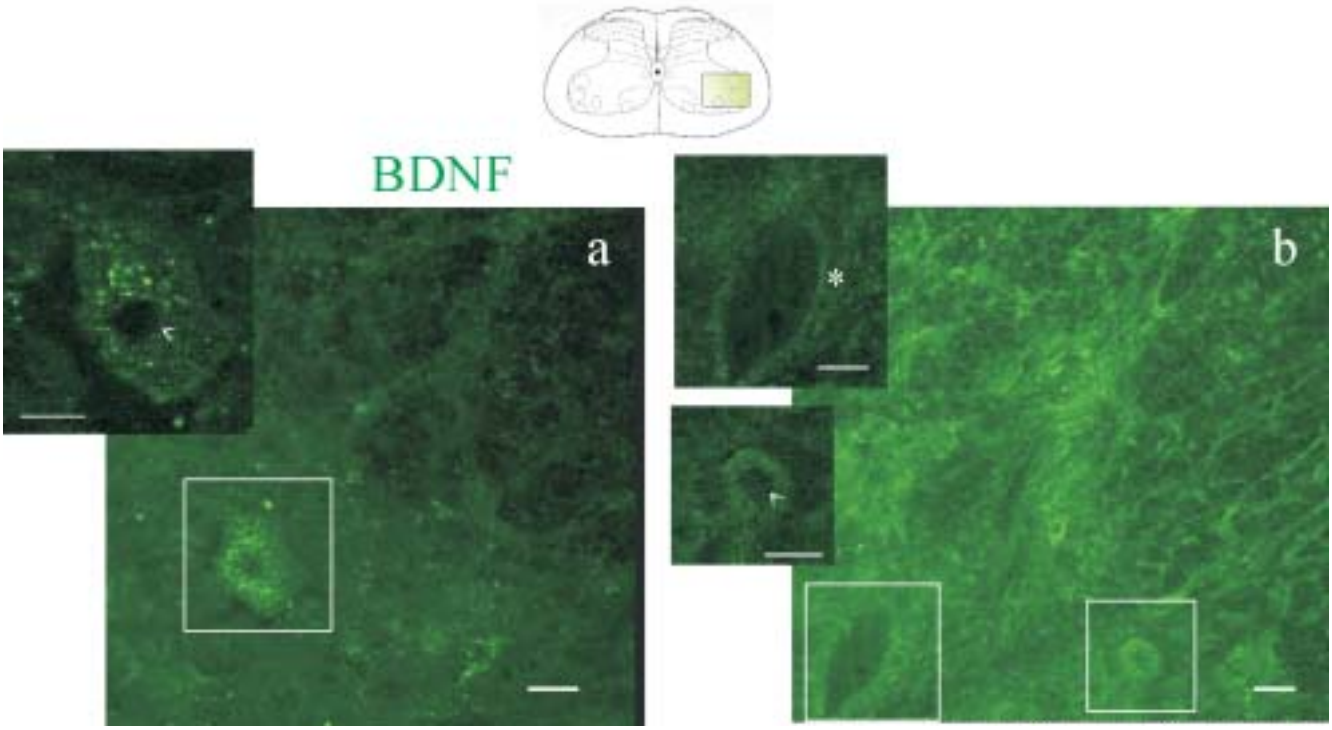

Fig. 1. Confocal microscopy analysis of the distribution of BDNF immunofluorescence (IF) in the L3 segment of the lumbar spinal cord of control (non exercised) (a) and of 7 days exercised (b) rat. At the top -the framed area on a scheme marks the ventral horn region, which underwent analysis. (a) In non exercised animal BDNF IF predominates in perikarya. Inset exemplifies large diameter neuron revealing presence of BDNF in large deposits in the paracentral part of cell body. (b) In the exercised animal an enhancement of BDNF IF in the neuropil and a dispersion of the IF deposits in perikarya which form an IF ring in the proximity of cellular membrane is demonstrated (asterisk). Nuclei devoid of IF are highlighted by white arrowheads. Fourteen scans have been superimposed to create the main pictures. Insets represent 1 μm single scan of the framed areas. Scale bar: 20 μm.

of Lamina IX, which may partly correspond to motoneurons. Short-term (ST) locomotor exercise led to an enhancement of BDNF staining in the fibers of gray and white matter, confirming our earlier observations after long-term exercise (LT). However, no clear effect of ST on BDNF IF level in neuronal perikarya was seen, contrary to that described LT exercise (Skup et al. 2002). A detailed analysis of single scans showed that in control animals IF deposits in large cells formed aggregates which accumulated mainly in the paracentral cytoplasm of the perikarya, surrounding the BDNF deposit-free karyoplasm (Fig. 1a). In exercised animals a dispersion of BDNF IF aggregates and redistribution of IF deposits from the paracentral part of perikarya towards their periphery and to the fibers was detected in some cells (Fig. 1b).

Analysis of $TrkB^{FL}$ IF in control rats (Fig. 2a) showed that $TrkB^{FL}$ is present in a cytoplasm of large neurons and in a number of small non-neuronal cells in their proximity (Fig 2). These small cells were previously identified as oligodendrocytes (Skup et al. 2002). The non-neuronal phenotype of these cells was confirmed in this study (exemplified in Fig. 3 for 28 days exercised rat). $TrkB^{FL}$ IF distribution repeated the pattern of staining obtained by us in former experiments with the use of bright field and fluorescent microscopy (Skup et al. 2002). The analysis of single scans disclosed an accumulation of $TrkB^{FL}$ IF deposits around neuronal nuclei, which were free of $TrkB^{FL}$ IF (Fig. 2a). It revealed also that fibers of some $TrkB^{FL}$ -positive oligodendrocytes contain $TrkB^{FL}$ IF deposits accumulated in varicosities (Fig. 2a). ST exercise led to an increase of both the number and intensity of $TrkB^{FL}$ IF in small cells as compared to non-trained animals (Fig. 2). The latter effect was comparable to the one caused by the LT exercise (Fig. 2 in Skup et al. 2002). Single scan

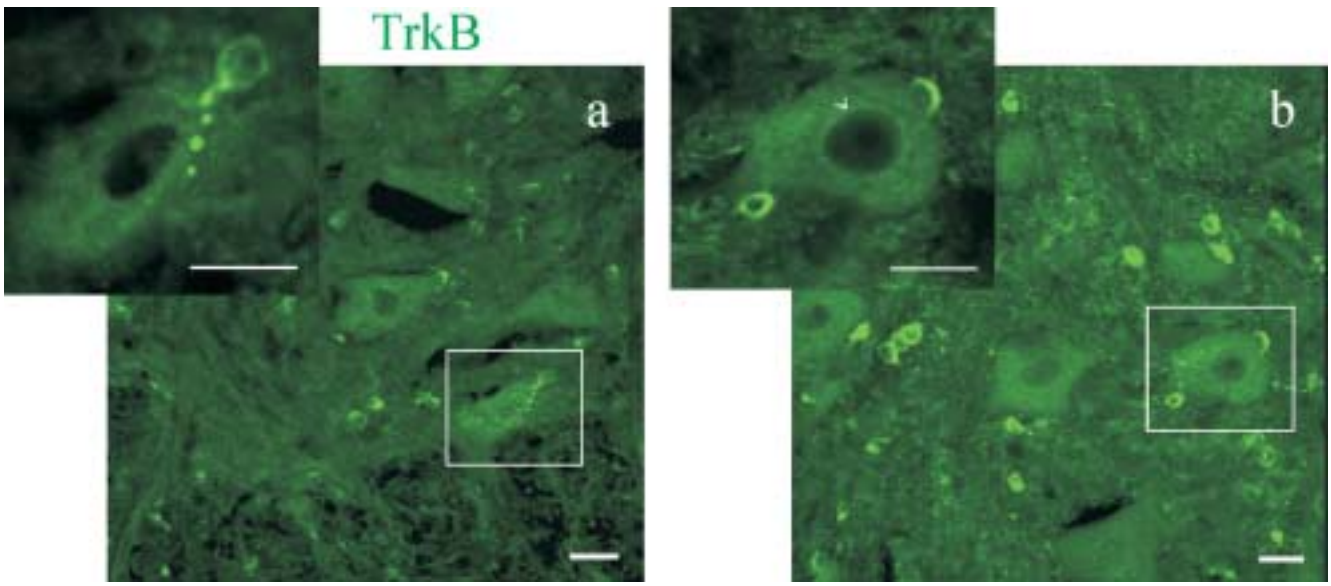

Fig. 2. Distribution of $TrkB^{FL}$ immunofluorescence (IF) in the L3 segment of the lumbar spinal cord of non exercised (a) and 7 days exercised (b) rat revealed with confocal microscopy. Note that both in control and exercised animal $TrkB^{FL}$ IF is present in large diameter neurons and in small cells. In the exercised animal the number of $TrkB^{FL}$ IF small cells is increased. Insets show large diameter neurons, which are in close apposition to small cells. The presence of $TrkB^{FL}$ IF varicosities in the fibers of a small cell is visualized (2a inset). Nuclei devoid of IF are clearly seen. Fourteen scans have been superimposed to create the main pictures. Insets represent 1 μm single scan of the framed areas. Scale bar: 20 μm.

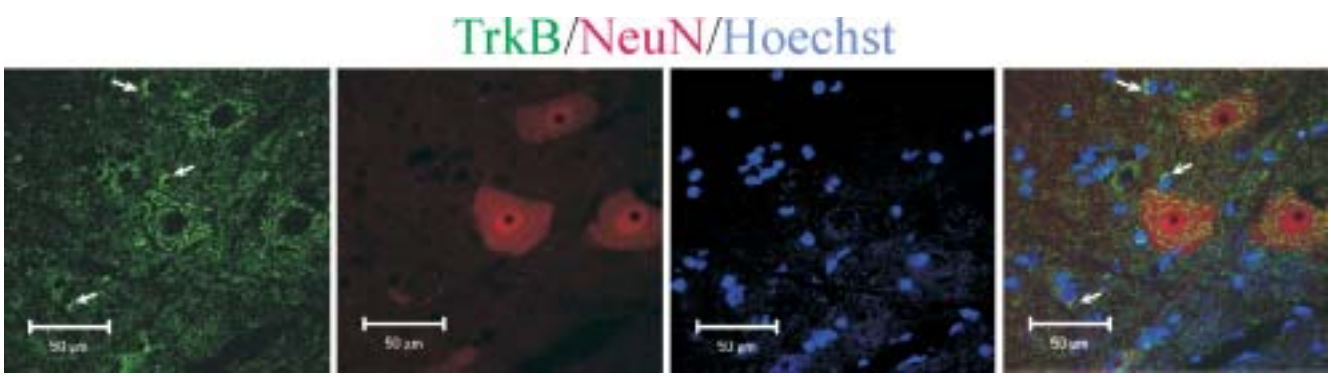

Fig. 3. Phenotypic identification of $TrkB^{FL}$ IF cells by labeling with neuronal marker NeuN in the rat subjected to long-term (28 days) locomotor exercise. Sections were co-stained with Hoechst dye to visualize cell nuclei. Note colocalization of $TrkB^{FL}$ /NeuN in large cells and strong $TrkB^{FL}$ IF in small cells, devoid of NeuN staining (arrows). Photomicrographs represent single scans of confocal microscopy images. The right photograph shows TrkB, NeuN and Hoechst staining merged.

analysis showed that oligodendrocytic fibers, which overlap with motoneuron like cells are in apposition to their surface, similarly in control and exercised rats (Figs. 2 and 3).

The main observation of our study is that seven days of moderate locomotor exercise is sufficient to cause an increase of BDNF protein expression in neurons of the lumbar spinal cord, but the intracellular pattern of BDNF IF upregulation is different from that found after long-term locomotor exercise (Skup et al. 2002). Namely, in the ST trained rats, BDNF IF signal in neurons, evaluated with assistance of confocal microscopy, was increased in fibers and dispersed in the pericentral part of perikarya, while in the LT trained rats a BDNF increase was detected in both cellular compartments (Skup et al. 2002). This result, together with the data

which document an early increase of BDNFmRNA and protein in the brain and spinal cord due to the training suggests an early onset of BDNF upregulation which may be not only sustained but also intensified by LT (Gomez-Pinilla et al. 2001, Macias et al. 2002, Skup et al. 2002). Different pattern of BDNF upregulation due to ST may be interpreted as a result of BDNF redistribution from perikarya to fibers. The antero- and retrograde transport of BDNF are well documented hence the intensification of BDNF intracellular transport may cause this redistribution (Aloyz et al. 1999, Altar et al. 1997, Fawcett et al. 2000). In our former study we showed that in the lumbar spinal segments many fibers labeled for presence of BDNF are dendrites (Skup et al. 2002). It was revealed by costaining with antibody against MAP2, which is known to exist in somatodendritic region (Garner et al. 1988, Kwak and Matus 1988). Thus it is possible that BDNF enhancement occurs in dendrites and is partly due to local dendritic synthesis, documented recently for proteins involved in regulation of synaptic activity (Pierce et al. 2000, Schuman 1999, Sutton et al. 2004, Tongiorgi et al. 2004). The presence of BDNFmRNA in dendrites (Dugich-Djordjevic et al. 1992, Tongiorgi et al. 2004) makes this hypothesis feasible, although activity-dependent axonal transport cannot be excluded (Kohara et al. 2001). Another important result of this study is that ST is effective in an enhancement of $TrkB^{FL}$ IF in the spinal cord. The increase occurred in small non-neuronal cells, mostly oligodendrocytes, as documented in our previous study (Skup et al. 2002). The pattern of an increase of $TrkB^{FL}$ receptor protein staining was the same as that resulting from the LT training. To our knowledge, this is the first report on the effectiveness of short-term exercise in upregulation of $TrkB^{FL}$ receptor in the spinal cord. This observation is in line with the data on $TrkB^{FL}$ mRNA upregulation found in the hippocampus following short-term training (Molteni et al. 2002). Based on our preliminary data which show no $TrkB^{FL}$ mRNA enhancement following LT training (Macias et al. 2003) we postulate that in the spinal cord there is a fast onset of the receptor mRNA and protein increase leading to subsequent maintenance of increased $TrkB^{FL}$ protein due to extended duration of exercise. The study revealed also that fibers of some $TrkB^{FL}$ positive oligodendrocytes are in very close apposition to the neurons. This implicates direct neuronal-oligodendroglial interactions mediated by BDNF neurotrophin (Lin and Bergles 2004). Altogether our study revealed new details of BDNF and TrkB cellular responses to exercise pointing to possible hot spots of BDNF action.

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