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The impact of training and neurotrophins on functional recovery after complete spinal cord transection: cellular and molecular mechanisms contributing to motor improvement

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Beneficial effects of locomotor training on the functional recovery after complete transection of the spinal cord indicate that in chronic spinal animals spontaneous recovery processes are enhanced and shaped by the training. The mechanisms of that use-dependent improvement are still not fully understood. This review tackles three aspects of this issue: (1) neurochemical attributes of functional improvement showing that concentrations of excitatory and inhibitory amino acids in the lumbar spinal segments, which were changed after transection, normalize after the training, or even raise beyond normal. As it does not translate to functional equilibrium between excitatory and inhibitory neurotransmission and may lead to hyperexcitability, the postsynaptic mechanisms which might be responsible for the hyperexcitability are discussed, including (i) dysfunction of K^+-Cl^- cotransporter KCC2, which controls the strength and robustness of inhibition, and (ii) altered function of 5-HT2 receptors, which may be targeted to restore KCC2 activity and intrinsic inhibition; (2) morphological changes of lumbar motoneurons and their inputs related to functional improvement of spinal animals, pointing to use-dependent diminution/reversal of the atrophy of the dendritic tree of the hindlimb motoneurons and of their synaptic impoverishment, which in paraplegic animals differs depending on the degree of disuse of the muscles; (3) the role of neurotrophins in motor improvement of spinal animals showing, that increases in neurotrophins due to training or due to efficient viral vector-based transgene expression, that might be responsible for the enrichment of the dendritic tree, elongation of processes and influence neurotransmitter systems in the areas subjected to plastic modifications after injury, correlate with improvement of locomotor functions.

Key words: spinal cord transection, locomotor training, excitatory/inhibitory amino acids, motoneuron innervation, KCC2 transporter, neurotrophins

INTRODUCTION

After complete transection of the spinal cord animals show the ability to perform stepping movements. This observation was reported by Sherrington in 1910 (Sherrington 1910) but it took quite a long time to prove that these abilities, representing spontaneous plastic changes in spinal neuronal networks, might be further shaped by motor exercise to improve locomotion.

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Using kinematic and EMG analysis Barbeau and Rossignol demonstrated that after complete transection of the spinal cord at the 13th thoracic segment, adult cats, subjected to locomotor training on a moving treadmill (time: 3 weeks–3 months–one year), were able to walk on the plantar aspect of the feet supporting their hindquarters and to perform well coordinated hindlimb movements with the cycle duration resembling that of intact cats walking at the same speed (Barbeau and Rossignol 1987). However, the mechanisms of this use-dependent functional improvement are still not fully understood. In this review we address the following questions concerning the mechanisms of

use-dependent improvement of motor abilities of chronic spinal animals: (1) What are the neurochemical attributes of that improvement; (2) What are the cellular changes that account for the use-dependent motor improvement of spinal animals; (3) What are the roles of neurotrophins, in particular of BDNF, in the use-dependent motor improvement of spinal animals?

NEUROCHEMICAL CHANGES IN NEUROTRANSMITTERS AND THEIR RECEPTORS IN THE THORACO-LUMBO-SACRAL SEGMENTS AFTER COMPLETE TRANSECTION OF THE SPINAL CORD

To generate stepping after a complete spinal cord transection, lumbar spinal circuitries have to adapt to the loss of supraspinal inputs. This adaptation involves functional reorganization, as demonstrated at the behavioral, biochemical, structural, and electrophysiological levels (Tillakaratne et al. 2000, 2002, Rossignol et al. 2004, Kitzman 2006, Petruska et al. 2007, Naito et al. 2008, Ichiyama et al. 2011). However, it is still not clear to which extent changes in neurotransmitter levels contribute to this reorganization, particularly in chronic spinal states.

In adult rats concentration of all neuroactive amino acids and monoamines, measured by means of HPLC in the whole tissue homogenate of the intact spinal cord, shows intersegmental rostro-caudal gradients, progressing caudally, with the highest concentrations among all tested compounds being glutamate (Glu) and glycine (Gly) (Skup et al. 2007). Within minutes after spinalization extracellular levels of excitatory amino acids, Glu and aspartate (Asp), are increased and contribute to neuronal damage (Panter et al. 1990, Farooque et al. 1996). Within hours tissue levels of these amino-acids, together with inhibitory ones, gamma-aminobutyric acid (GABA) and Gly, in injured spinal cord decrease, but GABA and Gly recover thereafter (Demediuk et al. 1989, Diaz-Ruiz et al. 2007).

In the chronic phase of changes after spinal cord transection in adult animals, the majority of studies on excitatory and inhibitory neurotransmission shows a profound decrease in neurotransmitters and neurotransmitter-related molecules, or reveals no significant changes, depending on the spinal region and segment below the lesion under analysis. Three weeks after complete transection of the spinal cord at low thoracic segments in adult cat, the concentration of Glu and Gly in L6–L7 segments decreased in the dorsolateral funiculus and tended to decrease in other areas of the spinal cord, whereas Asp and GABA concentrations were not affected (Rizzoli 1968). Six weeks after spinalization in adult rat the concentrations of both excitatory and inhibitory neurotransmitters decreased, particularly in the lesion site and in the rostral lumbar (L1–L2) segments, whereas they remained close to normal in the caudal lumbar (L3–6) segments where the majority of motoneurons innervating hindlimb muscles are located (Fig. 1). The impairment in the rostral lumbar segments involves also enzymes and neurotransmitter transporting systems. Caudally, more benign impairment of these systems take place (Ziemlińska et al. 2014).

Spinal cord transection causes also dramatic changes in descending neurotransmitter systems, depriving segments caudal to the lesion site of the modulatory effects of Glu and monoamines, as described for rabbits, dogs and rats (Stanton et al. 1975, Hadjiconstantinou et al. 1984). In adult rats, one month (Magnusson 1973, Roudet et al. 1994) and six weeks (Skup et al. unpublished) post-transection, a small amount (less than 2%) of noradrenaline (NA) was reported to remain in the caudal stump of the spinal cord. Because seven months after a complete spinal cord transection noradrenergic axons, suggested to derive from peripheral sympathetic neurons, have been detected caudal to a transection site, they may provide this low level of NA (Takeoka et al. 2010). Also serotonin (5-HT) concentrations reveal residual levels, not exceeding 2% of controls (Ziemlińska et al. 2014), but in this case residual 5-HT may be prescribed to a limited number of serotonergic interneurons, reported to persist in adult rats (Newton et al. 1986, Newton and Hamill 1988, Kubasak et al. 2008, Takeoka et al. 2009, Kong et al. 2010).

The effects of spinal cord transection on the locomotor functions and changes in neurotransmitter systems were also extensively investigated in young adult rats receiving spinal cord transection at mid-thoracic segments as neonates (Robinson and Goldberger 1986, Tillakaratne et al. 2010, Cantoria et al. 2011), for review see (Murray et al. 2004). These animals spontaneously recovered higher stepping abilities than rats transected as adults and their improvement was attributed to the reorganization of the lumbosacral neuronal network as there was no regeneration detected across the site of injury (Robinson and Goldberger, 1986, Tillakaratne et al. 2010). In rats transected as neonates and tested as

young adults by means of HPLC, the concentrations of Glu and Gly in the lumbar segments were not significantly different from that in control group (Cantoria et al. 2011), being in line with our observations on caudal lumbar segments in rats transected as adults.

Importantly for our understanding of the inhibitory transmission in spinal rats, not only Gly but also the levels of GABA, GAD67 and GAD65 transcripts of GABA synthesizing enzymes, were not significantly different from those in the intact rats in the caudal lumbar segments of spinal rats transected as adults (Ziemlińska et al. 2014). These observations strongly suggest that the level of inhibition in the L3–L6 networks is similar in chronic spinal and intact rats, leading us to question the proposition by Tillakaratne and coauthors (2002) that glycinergic and GABAergic inhibition is increased in the caudal lumbar segments. Moreover, in chronic spinal animals the levels of both excitatory and inhibitory neuroactive amino acids are in relative equilibrium in the lumbar segments although their concentration is higher in the low lumbar (L3–L6) than in L1–L2 segments (Ziemlińska et al. 2014).

Yet there are number of studies showing that excitatory drive to motoneurons prevails in chronic spinal animals leading to episodes of clonic and jerk movements, symptoms of spasticity (Wainberg et al. 1990, Sheean 2002). An important explanation for reduced spinal inhibition in the weeks after SCI stems from the study by Vinay's group (Boulenguez et al. 2010) who showed the postinjury decrease in the spinal level of the neuron-specific potassium-chloride cotransporter (KCC2), which is a neuronal extruder of Cl^- ions, associated with ionotropic GABA(A) and glycinergic (GlyR) receptor function. The proper functioning of this molecule contributes to the potential of a neuron to hyperpolarize. Vinay's group showed a decrease of KCC2 cotransporter in the ventral horn of spinal rats (Boulenguez et al. 2010), which was confirmed in our study (Ziemlińska et al. 2014). Such a decrease might reduce the generation of Cl^--dependent hyperpolarizing postsynaptic currents mediated by GABA(A) and glycine receptors (Thompson and Gahwiler 1989, Kaila 1994, Payne et al. 2003). It contributes to depolarization instead of hyperpolarization (Toyoda et al. 2003, Coull et al. 2005), leading to increased excitability of the spinal network. Therefore, the relative equilibrium in the concentration of excitatory and inhibitory neuroactive amino acids detected in L3–L6 neuronal network of the spinal animals (Ziemlińska et al. 2014) does not translate to functional equilibrium between excitatory and inhibitory neurotransmission as the latter is controlled by an adequate level and function of KCC2 cotransporter.

With this respect, an important mechanism for regulating the motoneuron excitability, discovered recently in the sacral segments of the spinal cord after transection at rostral sacral segments, has to be taken into account. It involves 5-HT_{2B} and 5-HT_{2C} receptors, abundant in motoneurons of the chronic spinal animals, which might be active in the absence of their ligand and become constitutively active after injury (Murray et al. 2010, 2011). Constitutive activity of these receptors restores persistent inward calcium and sodium currents (Ca and Na PIC) that are normally present in spinal interneurons (Dai and Jordan 2010), and are crucial for enabling motoneurons to produce sustained motoneuron discharges in response to synaptic inputs and lead

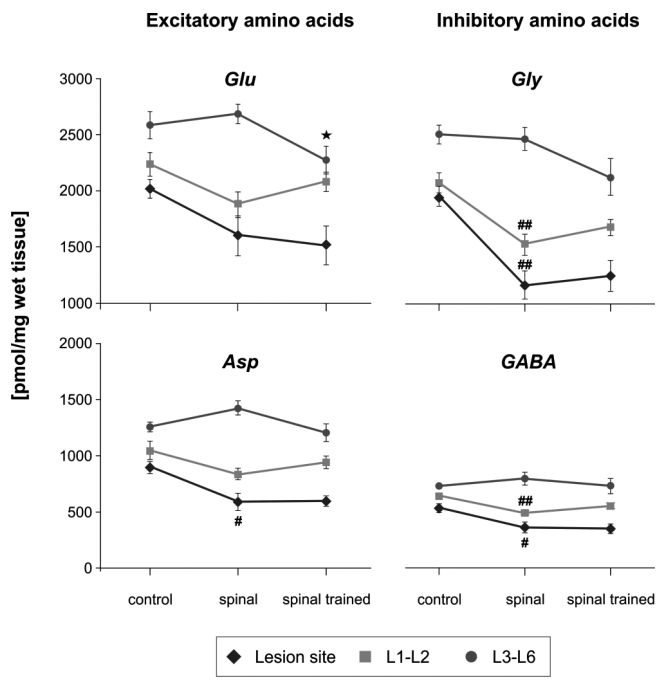

Fig. 1. Concentration of Glu, Asp, Gly and GABA in the caudal thoracic (lesion site), rostral lumbar (L1–L2) and caudal lumbar (L3–L6) segments in intact (control), spinal and spinal trained rats (5 weeks of treadmill locomotor training, up to six 4 min walking sessions daily separated by 30 min rest). The HPLC method was used. The data are expressed as the mean ± SEM. Four adult rats were tested in each group. *T*-Student Test for independent samples was applied here; significant differences between spinal rats and intact controls were marked with $^{\#}P<0.05$, $^{\#\#}P<0.01$, and those between spinal and spinal trained groups are indicated by $^{*}P<0.05$.

to sustained muscle contractions (Li et al. 2004, Harvey et al. 2006, Button et al. 2008, Murray et al. 2010). This issue and the effect of upregulation of 5-HT_{2C} on the PICs in motoneurons of chronic spinal animals have been extensively discussed in the very recent review by D'Amico and colleagues (2014). Here we point to another aspect of the functional consequences of constitutive activity of 5-$HT_{2B/2C}$ receptors, which links them to KCC2. Namely, as shown by Bos and coauthors (2013), activation of these receptors induces a depolarizing shift of reversal potential E_{IPSP}, whereas blocking them hyperpolarizes E_{IPSP}, indicating that activation of 5-$HT_{2B/2C}$ receptors contributes to down-regulation of KCC2 activity. Also recently 5-HT_{2A} receptors emerged as important factors for the maintenance of equilibrium between excitation and inhibition of motoneurons, as their activation was shown to restore cell membrane expression of KCC2 (Bos et al. 2013), restore endogenous inhibition and reduce spasticity after spinal cord transection at low-thoracic segments.

A recent study by D'Amico and others (2013) indicates that in humans, with severe spinal cord injury, facilitation of PICs and muscle spasms mainly occur due to constitutive $5HT_2$ and noradrenergic alpha 1 receptor activity. The mechanisms described above might, therefore, be involved in the phenomenon of hyperexcitability not only in spinal animals but also in humans (Kiehn and Eken 1997, Gorassini et al. 1998, Li et al. 2004, D'Amico et al. 2013, Ren et al. 2013).

We strongly support the concept that within the thoraco-lumbar segments, the crucial factors, which influence the functionality of motoneurons, may be other than in the sacral segments. As discussed by Navarett and coworkers (2012) in contrast to the sacral region which has a limited repertoire of internal motor circuits (albeit receives both glutamatergic and cholinergic inputs), higher regions of the spinal cord contain intricate networks of regulatory interneurons, e.g. the glutamatergic (VGluT2-positive) neurons that comprise the locomotor central pattern generator (CPG) that regulates the rhythm of muscle contraction (Kiehn 2006). Glutamate, along with serotonin and acetylcholine, promotes plateau potentials in motoneurons by enhancing the activity of L-type calcium channels, thus generating the PICs that depolarize the membrane. When extracellular serotonin is depleted following a transection in Th10 segment, and glutamatergic neurotransmission from the preserved CPG interneurons is sustained, as shown for VGluT2 expression in conditions of BDNF overproduction, described next (Ziemlińska et al. 2014), it may provide enough excitatory input to maintain the depolarized state of motoneurons. Moreover, recently demonstrated functional link of 5-HT_{2C} receptors and NMDA receptors which, by formation of a complex, lead to enhancement of NMDA depolarization, points to the possibility, that even basal level of constitutive 5-HT_{2C} activity may be sufficient to increase Glu-mediated signaling.

The changed excitability of the spinal network after spinalization is a phenomenon shown to engage numerous glutamatergic and GABAergic neurotransmission-related molecules. Wienecke and coworkers (Wienecke et al. 2010) showed that after transection of the caudal spinal cord the NMDA receptor complex is up-regulated while the GABA(A) receptor is down-regulated. Complete midthoracic transection at P5 results in a long-term deficit of GABA(A) γ2 subunit levels in the soleus motoneurons (Khristy et al. 2009), suggesting impairment of GABA signaling in this motoneuron pool. These data indicate the complexity of the mechanisms which lead to altered motoneuron excitability.

ALTERED INPUTS AND MORPHOLOGICAL CHANGES OF LUMBAR MOTONEURONS AFTER COMPLETE TRANSECTION OF THE SPINAL CORD

Deprived of supraspinal input, spinal neuronal networks are subjected to spontaneous rearrangement as illustrated by the experiments of Gazula and coworkers (2004) who injected viral constructs of β-galactosidase into the sciatic nerve to label the dendritic tree of hindlimb motoneurons. The authors analyzed morphological changes of the dendritic tree of motoneurons after complete spinal cord transection showing that it undergoes atrophy. Similar observations were reported by Kitzman, who showed an atrophy of the dendritic tree of the tail motoneurons after complete transection of the spinal cord at rostral sacral segments (Kitzman 2005, 2006).

As these morphological changes would influence inputs on motoneurons limiting the number of synapses apposing them, several groups, including our own addressed the question whether spinal cord transection causes a reduction in the number of synaptic boutons on motoneurons (Kitzman 2006, Skup et al. 2012). The assumption of a decreased density of synaptic boutons apposing perikarya and proximal

dendrites of motoneurons after spinal cord transection was found to be true with respect to some but not all neuron-type specific inputs and motoneuronal populations (Kitzman 2006, Skup et al. 2012). Six weeks after complete transection of the spinal cord at low thoracic segments total synaptic coverage of the soma of motoneurons innervating the hindlimb muscles decreased, as indicated with the synaptophysin immunolabeling (Macias et al. 2009). In line with that observation, five weeks after complete spinal cord transection, the synaptophysin mRNA level, measured in L1–L2 and L3–L6 segments was downregulated (Fig. 2) (Płatek 2014). These observations indicate a decreased potential of motoneurons, but also interneurons, to produce this protein and suggest a contribution of the presynaptic component to altered responses of motoneurons, which might be expected owing to the profound changes in their morphology. A decrease of synaptophysin expression around the hindlimb motoneurons was also reported by de Leon and coauthors (2011) in adult rats spinalized as early neonates. However, in the same experimental model, the synaptic coverage of tibialis anterior motoneurons did not differ compared to the intact adults in the synaptic input to α-motoneurons (Ichiyama et al. 2011).

Which inputs contribute to deficits of synaptophysin "coverage" of motoneurons? Obviously, a significant portion of these deficits derive from reduced supraspinal inputs. Also relatively large cholinergic C-boutons, terminating predominantly on the soma and proximal dendrites of α-motoneurons, if reduced in number after spinal cord transection, could contribute to the decrease of a total synaptic density on the hindlimb motoneurons and might allow differentiation of the effect of the spinalization on different motoneuronal pools (Macias et al. 2009, de Leon et al. 2011). Cholinergic innervation of α-motoneurons was intensively investigated by several groups during the last few years (Wilson et al. 2004, Miles et al. 2007, Zagoraiou et al. 2009, Stepien et al. 2010). It was demonstrated that the only source of cholinergic C boutons innervating α-motoneurons originate from a discrete group of interneurons located lateral to the central canal, the medial partition $V0_c$ neurons (Miles et al. 2007, Zagoraiou et al. 2009). Cholinergic activation of C-boutons, acting *via* m_2 muscarinic receptors, increases excitability of motoneurons by reducing the action potential afterhyperpolarization (AHP) (Miles et al. 2007) and this modulation might be regulated in a task-dependent manner (Zagoraiou et al. 2009).

To verify the effect of spinal cord transection on cholinergic innervation of α-motoneurons in our recent study we focused on two groups of motoneurons innervating muscles acting antagonistically at the ankle joint: tibialis anterior (TA), the ankle flexor, and soleus (Sol), the ankle extensor, which were prelabeled (Skup et al. 2012). These two muscles are subjected to different conditions in paraplegic rats: during dragging the paw on its dorsal aspect TA is providing a source of proprioceptive stimulation of motoneurons. In contrast, Sol muscle, which is unloaded, deprives motoneurons of such stimulation. Profound difference in the daily duration of the Sol muscle activity, which is approximately 7 times longer than that of the TA muscle (Hensbergen and Kernell 1997, Roy et al. 2007) also suggested that the effect of chronic disuse might differ in these two groups of motoneurons. A decrease in the weight of the extensor but not of the flexor muscles operating at the ankle joint of spinal animals (Alaimo et al. 1984, Roy et al. 1999, Talmadge et al. 2002) and the observation that mechanical and biochemical characteristics of the TA muscle in the cat were much less affected by a complete transection of the spinal cord than of the medial gastrocnemius, a synergist of the Sol muscle (Roy et al. 1999), supported this possibility.

Indeed, the complete transection of the spinal cord at low thoracic segments caused a profound reduction in the number of VAChT IF boutons apposing the soma membrane of Sol but not TA motoneurons. It indicates

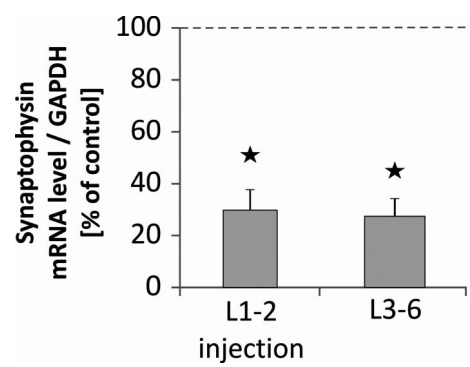

Fig. 2. Changes of synaptophysin transcripts in the lumbar segments of the rat spinal cord, 5 weeks after spinalization and AAV5-EGFP transduction. The data are expressed as the Mean ± SD. Four intact (control) and five spinal AAV-EGFP injected rats were used here. Mann-Whitney *U* Test was used to analyze the differences between the control and AAV5-EGFP transducted animals (**P*<0.05).

that the lesion exerts different effect on the innervation of motoneurons which differ functionally, showing pronounced effect of disuse on the extensor but not on the flexor motoneurons acting at the ankle joint (Skup et al. 2012). These results also show that decreased number of cholinergic terminals clearly contributed to the deficit of synaptophysin observed after spinal cord transection. In line with the data by Miles and colleagues (2007, Petruska et al. 2007), it could also affect the AHP, leading to a decrease of motoneuron excitability.

In line with these observations, after complete transection of the spinal cord at the rostral sacral segments, the number of synaptic cholinergic boutons (immunolabeled for the vesicular transporter of acetylcholine – VAChT) apposing the tail motoneurons, drastically decreased and 12 weeks later dropped practically to zero (Kitzman 2006). In contrast, the number of synaptic boutons immunolabeled for the glutamate (VGluT2) or GABA (VGAT) vesicular transporters either increased (VGluT2) or did not change in the number (VGAT) after that surgery, whereas a profound deficit in the level of VGluT2 mRNA was detected in the rostral lumbar segments with similar tendency in L3–L6 segments. The latter suggests that there is an interneuronal dysfunction and a decrease of excitatory drive from glutamatergic interneurons in the lumbar circuits after complete spinal cord transection at low-thoracic segments (Ziemlińska et al. 2014). The comparison of responses of glutamatergic inputs at the lumbar and sacral segments suggests that glutamatergic transmission to hindlimb motoneurons is more vulnerable to the spinal cord transection than that to the tail motoneurons and that the overinnervation by the latter might contribute to the tail spasticity.

LOCOMOTOR TRAINING CHANGES INPUTS AND RECEPTIVENESS OF MOTONEURONS TO EXCITATORY AND INHIBITORY STIMULI AFTER SPINAL CORD TRANSECTION

The beneficial effects of locomotor training on a broad range of functions, from motor to intellectual and memory, raised questions about their molecular mechanisms.

As already mentioned, when compared with the intact animals, motoneurons from spinal animals display marked atrophy, with loss of dendritic membrane and elimination of branching (Gazula et al. 2004, Ilha et al. 2011). None of these regressive changes in morphology were found in the motoneurons from spinal animals that underwent exercise. This alleviation of impoverishment in the dendritic tree and an increased number of synaptic boutons terminating upon motoneurons, observed in spinal animals subjected to locomotor training (Macias et al. 2009, Skup et al. 2012), may be expected to contribute to improved functioning of the motoneuron in these conditions. Also in rats spinally transected at 5 days of age, and subjected as young adults to 4 weeks of weight-supported treadmill locomotor training, the expression of synaptophysin on the hindlimb motoneurons, which decreased after injury, increased in animals performing the largest number of steps, i.e., 1000 steps, in the daily training (de Leon et al. 2011). Cholinergic innervation of motoneurons, controlling the ankle extensor and flexor muscles, was also influenced by locomotor training (Skup et al. 2012). After five weeks of treadmill locomotor training of adult spinal rats, cholinergic input to the Sol motoneurons, which profoundly decreased after injury, partly recovered (Skup et al. 2012). Together with observations that locomotor training of spinal animals restored AHP depth values, in the ankle extensor motoneurons of rats transected as neonates and trained as young adults (Petruska et al. 2007) these data point to two important aspects which contribute to improvement of locomotor functions.

The invention of laser capture microdissection (Emmert-Buck et al. 1996), a useful albeit challenging method of collecting selected cells for DNA, RNA and protein analysis from microscopic regions, opened new possibilities for studies of altered gene expression also at the level of single motoneurons in the spinal cord. It revealed that, in response to spinal cord injury, the regulation of excitatory and inhibitory signal transduction in motoneurons shifted in the balance toward increased excitability, where the genes encoding glutamatergic N-methyl-d-aspartate receptor complex together with cholinergic system are up-regulated and the genes encoding gamma-aminobutyric acid type A receptor (GABA(A)R) subunits are down-regulated (Wienecke et al. 2010). Also, the expression levels of other molecules indicate, that a significant change of motoneuron properties take place after spinalization. Genes regulating both calcium and sodium channels, responsible for inward currents, revealed a complex regulation, and over half of the genes coding for potas-

sium channels, responsible for regulation of outward currents, were down-regulated (Wienecke et al. 2010). In light of the further discussion it is worth to point to one of these genes, the gene *Kcnn2*, encoding a small-conductance calcium-activated potassium channel protein (SK2), which generates the medium component of the afterhyperpolarization (mAHP) following action potentials in motor neurons. This conductance is blocked by serotonin in motoneurons, leading to increased expression of plateau potentials (Hounsgaard and Kiehn 1989, Grunnet et al. 2004). As suggested by Wienecke and colleagues (2010), SK2 down-regulation after spinalization could therefore signify an increased ability to generate plateau potentials.

Laser capture microdissection allowed also identification of changes in the content of several mRNAs in lumbar motoneurons from rats previously subjected to normal cage activity and different exercise regimes for 7 days and 16 weeks (Woodrow et al. 2013). As a result of the prolonged daily treadmill training, significant decreases in mRNA contents were evident for specific receptors: 5-hydroxytryptamine receptor 1A (5-HT_{1A}), GABA(A) receptor subunit alpha 2 (GABA(A)α2), and for the SK2 channels in the motoneurons from 16-week-trained rats. The reduction in GABA(A) receptors and SK2 channels, the latter being responsible not only for the mAHP but also reported to provide a negative feedback on NMDA signaling (Ngo-Anh et al. 2005), shows a change in the intrinsic properties of motoneurons and suggests a shift towards increased excitability. However, since the function of SK channels is under negative control of 5-HT_{1A} receptors (Grunnet et al. 2004), which also decrease after training, the net effect of the reported changes on motoneuron excitability is not clear.

These two sets of data suggest that locomotor training, if applied after spinalization, might further decrease motoneuron ability to respond to GABAergic signaling and lead to hyperexcitability. In line with this assumption are our recent results which showed, that also glycinergic signaling in the lumbar ventral horn in rats with complete spinal cord transection may be decreased after the training (Maciejewska 2013, Ziemlińska et al. 2013). Although we did not find a significant change in markers of glycinergic inputs, the plasmalemmal abundance in GlyR and gephyrin, a postsynaptic scaffolding protein, essential for the clustering of glycine and GABA(A) receptors at inhibitory synapses, were significantly decreased in motoneurons after long-term training.

Postsynaptic effects of locomotor training on molecular changes are not isolated from the presynaptic ones. Compared to controls, spinal rats have reduced synaptophysin protein expression and Na^+-, K^+-ATPase activity in lumbar spinal cord (Ilha et al. 2011) and reduced number of inputs to α-motoneurons and, as described earlier, reduced number of inputs (Macias et al. 2009), including cholinergic ones (Skup et al. 2012). In treadmill-trained spinal rats the size of motoneuronal perikarya and the level of synaptophysin expression and Na^+-, K^+-ATPase activity normalized providing an additional set of data showing that training can promote activity-dependent neural plasticity in the lumbar spinal cord, translating into functional improvements. Spinalization tended to reduce both GABA/Gly and Glu/Asp levels in the rostral lumbar segments, barely affecting their levels in the caudal lumbar segments (Ziemlińska et al. 2014). Locomotor training modulates their levels and the net effect of locomotor training on pre- and postsynaptic components of signaling results in functional improvement (Fig. 1).

An increase of GAD67 in the spinal cord, caudal to the lesion site, at low thoracic segment in the cat, and its normalization after locomotor training was first reported by the Edgerton group (Tillakaratne et al. 2002). This observation was not confirmed in the rat, where GABA concentration was evaluated in the whole-tissue homogenates using the HPLC method (Fig. 1). Five weeks of treadmill locomotor training of spinal animals tended to increase GABA concentration in L1–L2 segments but tended to decrease it in L3–L6 segments where the concentrations of Asp and Gly were also lowered compared to spinal non-trained rats (Fig. 1). The functional improvement observed in the spinal rats after the training was not accompanied by clear-cut changes in the concentration of both excitatory (Glu, Asp) and inhibitory (Gly, GABA) neuroactive amino acids in the lesion site (at low-thoracic segments) and in the lumbar segments (Fig. 1; see also Table III in Ziemlińska et al. 2014).

Interestingly, when rats that received spinal cord transection at 5 days of age were trained as young adults, by a robotic treadmill system with body weight support, both Glu and Gly concentrations in the lumbar segments were clearly higher than in intact (but not when compared to spinal untrained) rats (Cantoria et al. 2011). The level of these two neuroactive amino acids was in a positive correlation with the stepping performance (Cantoria et al. 2011). Together with the

observation that glutamatergic (VGluT1) and glycinergic inputs (VGlyT2) to motoneurons were strikingly increased in trained rats, as compared to untrained ones, it shows that spinal cord of young adults transected as neonates is clearly susceptible to the training, responding to it with significant reorganization of the spinal network.

Summing up, the atrophy of the dendritic tree of motoneurons and a decrease of the number of synaptic boutons terminating upon their soma and proximal dendrites observed after complete transection of the spinal cord and compensation of those deficits after locomotor training indicate profound morphological reorganization in the spinal neuronal network which occurs in activity-dependent manner. The observed course of changes of the synaptic innervation on motoneurons was different depending on: (1) neurotransmitter phenotype of terminals, (2) the diversity in the effects of a chronic disuse on functionally different groups of motoneurons, (3) segmental location of motoneurons.

INVOLVEMENT OF NEUROTROPHINS IN THE PLASTICITY OF SPINAL CIRCUITRIES AND FUNCTIONAL RECOVERY

Abundance of neurotrophins in the spinal cord

During the past 30 years, neurotrophins, and in particular brain-derived neurotrophic factor (BDNF), have emerged as serious candidates for linking synaptic activity and neuronal plasticity (Lessmann et al. 2003, Rose et al. 2004, Bramham and Messaoudi 2005, Nagappan and Lu 2005, Greenberg et al. 2009, Kuczewski et al. 2009). Neurotrophin expression and secretion are under control of activity-dependent mechanisms and, besides their classical role in supporting neuronal survival, they modulate nearly all key steps of network construction. In particular, the mature form of BDNF can serve as an axon terminal- or target- derived message, acting through the tropomyosine-related kinase B ($TrkB^{FL}$) high-affinity receptors, which transfer their signal through the cell membrane for development and activity-dependent synaptic plasticity at the single cell level. A fascinating area of research concerns the disclosure of the mechanisms which would explain the selectivity of their transduced signals. Local synthesis and secretion of BDNF at active synapses, $TrkB^{FL}$ receptor turnover and insertion in the plasma membrane and, the most intriguing, partitioning of neurotrophin receptors in synaptic rafts, membrane microdomains enriched in synaptic zones (Nagappan and Lu, 2005) are among the possibilities. Accumulated data show that both $TrkB^{FL}$ receptors and p75 low-affinity receptors mediating BDNF signaling localize in lipid rafts together with the effectors of their downstream signaling pathways (Higuchi et al. 2003, Suzuki et al. 2004, Pereira and Chao 2007). Which of these BDNF-dependent mechanisms operate in the spinal cord neurons has not been established yet.

Evidence has accumulated that BDNF is widely distributed in the spinal cord of the adult cat (Frisen et al. 1992, Rong et al. 2011) and rat (Dreyfus et al. 1999, Scarisbrick et al. 1999, Skup et al. 2002). The sites of action of spinal BDNF have been ascertained only partially. For instance, BDNF can be released by motoneuron dendrites or cell bodies and act retrogradely on interneurons, descending afferent axons or surrounding glia (Yan et al. 1993, Rind et al. 2005). Alternatively, BDNF transported anterogradely in motor axons affects distal targets, both skeletal muscle and perisynaptic Schwann cells, likely participating in activity-dependent modifications of neuromuscular transmission *per se* but not of contractile or fatigue properties of the muscle (Poo 2001, Cohen-Cory 2002, Mantilla et al. 2004). Importantly, motoneurons are not only a source of BDNF and NT-4 (neurotrophin-4, the other $TrkB^{FL}$ ligand) but the survival and repair of motoneurons strongly depend on these neurotrophins (Koliatsos et al. 1993, Friedman et al. 1995, Keeler et al. 2012). During development of the neuromuscular junction (NMJ) in Xenopus, NMJs formed on myocytes overexpressing NT-4 display greater spontaneous synaptic activity and enhanced evoked synaptic transmission compared to synapses formed on non-overexpressing myocytes when tested in a neuron-myocyte coculture (Wang et al. 1998). Accordingly, BDNF treatment rapidly potentiates synaptic activity at developing amphibian NMJ (Wang and Poo 1997). In adult rats and mice also skeletal muscles produce and release BDNF (Koliatsos et al. 1993, Fernyhough et al. 1995, Griesbeck et al. 1995, Dupont-Versteegden et al. 2004, Zhao et al. 2004, Roux et al. 2006, Garcia et al. 2010b, Gajewska-Woźniak et al. 2013), which may act in an autocrine fashion or exert retrograde effects *via* TrkB presynaptic receptors, modulating

ACh release (Garcia et al. 2010a). In an absence of a short-term effect of neurotrophins at the NMJ, the $TrkB^{FL}$ receptor seems to be active and coupled to ACh release by a process of muscarinic receptor- mediated transactivation (Lee and Chao 2001).

Spinal neurons and peripheral targets produce also a prototypical neurotrophic factor, nerve growth factor (NGF), which is strictly related to regulation of nociceptive transmission. Contrary to BDNF, NGF concentrations are very low in the normal spinal cord but increase at least 4-fold within a week after spinal cord injury (SCI) (Bakhit et al. 1991, Widenfalk et al. 2001, Beattie et al. 2002, Marsh et al. 2002, Murakami et al. 2002). Neurons, glial cells, meningeal cells and Schwann cells contribute to this increase (Brown et al. 2004). Although exogenous NGF can have trophic actions on sensory neurons after SCI (Tuszynski et al. 1996, Grill et al. 1997, Menei et al. 1998, Ramer et al. 2000, Romero et al. 2001), central sprouting of sensory fibers after spinal cord injury may lead to autonomic dysfunction (dysreflexia), pain, and apoptotic cell death, contributing to the pathology of spinal cord injury (Krenz et al. 1999, Romero et al. 2000, Jacob et al. 2001). Therefore NGF has not been used in experimental treatments of the spinal cord injury; on the contrary, treatment with TrkA-IgG to block NGF signal transmission through TrkA receptors was used, resulting in beneficial suppression of the development of autonomic dysreflexia after SCI (Marsh et al. 2002).

The fourth member of neurotrophin family, Neurotrophin-3 (NT-3), is also produced in the spinal cord, but its protein levels in the normal spinal cord of the rat, as compared to BDNF, are low (Gajewska-Wozniak et al. 2013). NT-3 which is mainly involved in control of functional efficiency of the proprioceptive systems, acting *via* TrkC receptors (see Patel et al. 2003, Gajewska-Wozniak et al. 2013) was found to respond in a complex way to the spinalization, electrical nerve stimulation and training (Gomez-Pinilla et al. 2001, Gajewska-Wozniak et al. 2013). NT-3 delivery was found to be less effective than BDNF delivery in bringing improvement of stepping behavior after complete spinal cord transection (Boyce et al. 2012). However, it is indispensable for the reinnervation processes after injury of peripheral nerves (Munson et al. 1997) and it is also important for anatomical reorganization and reduced functional deficit after injury of the cortico-spinal tract (Fortun et al. 2009).

Locomotor training differentially affects the expression of BDNF, NT-4 and their TrkB receptors in the spinal cord and muscles: levels of regulation

Both BDNF mRNA and protein levels in the lumbar enlargement are potently up-regulated by various exercise regimens that involve hindlimb stepping in uninjured (Gomez-Pinilla et al. 2001, 2002, Macias et al. 2002, 2005, 2007, Skup et al. 2002, Ying et al. 2006) and injured (Macias et al. 2009, Keeler et al. 2012, Houle and Côté 2013) rats. Evidence that in the intact rat, long-term locomotor treadmill exercise of moderate intensity stimulates spinal circuitries sufficiently to recruit the majority of lumbar spinal neurons to increased BDNF synthesis, disclosed the requirements of the whole lumbar network for increased neurotrophic support, to be up to intensified physical activity in the physiological range (Skup et al. 2002, Macias et al. 2009). The degree of mobilization of the circuitry to provide enhanced neurotrophin signal in these conditions is reflected by changes in the number of cells expressing BDNF transcripts. These were reported to rise in the population of cells of cross-sectional area from 200 to 1200 μm^2, depending on the cell-size, from 50–75% in non-exercised rats, to 80–90% of these cells in exercised rats. Moreover, the response of the largest motoneurons of cross-sectional area >1800 μm^2, the vast majority of which express BDNF, leads to significantly higher level of BDNF transcripts, which is matched by increased levels of BDNF protein (Skup et al. 2002, Macias et al. 2007). An important study by Ollivier-Lanvin and coauthors (2010) provided evidence, that exercise-related increase in spinal BDNF mRNA is dependent on maintenance of sensory information being transmitted during exercise; deafferentation which removes inputs arising from large-diameter proprioceptors abolishes exercise-dependent increase in BDNF expression (Ollivier-Lanvin et al. 2010). Whereas changes of BDNF expression concerns cells localized mostly in the grey matter, the same training also increases NT-4 neurotrophin in the white matter of the lumbar enlargement, pointing to the astrocytes as an additional source of neurotrophins signaling through the $TrkB^{FL}$ receptor (Skup et al. 2002). Thus, locomotor training may be considered a potent tool to enhance/intensify the multifaceted neurotrophin regulation of spinal circuitry.

In the muscle of adult rats BDNF was shown to undergo complex regulation in an activity-dependent mode, both at the mRNA and protein levels. For example, low-threshold continuous bursts of electrical stimuli that activate the group Ia afferents, led to a decrease of BDNF mRNA, accompanied by a decrease of TrkB mRNA expression in the soleus muscle (Gajewska-Woźniak et al. 2013). Five or more days of exercise increased BDNF mRNA expression in that muscle both in the intact (Gomez-Pinilla et al. 2001) and spinal (Dupont-Versteegden et al. 2004) rats. Recently, exercise was shown to up-regulate muscle BDNF mRNA also in rats with a nerve crush (Sartini et al. 2013). Thus, not only spinal cord neurons but also their peripheral targets produce and are under control of neurotrophins, which may be up-regulated by physical training.

What do we know about the mechanisms which translate/convert locomotor stimuli to altered BDNF levels?

We are far from complete knowledge on the epigenetic mechanisms which are known to maintain long-lasting gene expression programs, but there is increasing evidence that exercise has a powerful influence on biological adaptation and maintenance of new functional "gains" that engages epigenetic mechanisms to control gene function (Gomez-Pinilla et al. 2011). The expected persistence of the training effects prompted the studies aimed to evaluate the possibility that the effect of exercise on the regulation of BDNF could involve these mechanisms, promoting stable changes in gene function. Epigenetic mechanisms operate at several levels of regulation including chemical modification of the DNA molecule by adding methyl groups to the CpG dinucleotide sites (Razin 1998), or histone acetylation. Such modification regulates the binding of the different transcription regulators, both enhancers and repressors, and the transcription machinery to control the expression of specific genes. The studies on the modulation of *Bdnf* gene are extremely challenging, as this gene is comprised of at least eight distinct promoters (there are at least four promoters in the rat) that initiate transcription of multiple distinct mRNA transcripts (Aid et al. 2007). Through the use of alternative promoters, splicing and polyadenylation sites, at least 18 transcripts can be produced, but remarkably, each encodes an identical initial BDNF protein product. It is hypothesized that it provides multiple layers of regulation, through alternative promoter usage, differential mRNA stability, or differential subcellular localization of either mRNA or protein (for review see Feng et al. 2007). It has been shown that transcription involving promoter IV, which is responsive to neuronal activity and can mediate synapse plasticity, is indeed subjected to epigenetic regulation. Promoter IV is suppressed by methyl-CpG-binding protein (MeCP2), which belongs to the family of methyl citosine-binding proteins that contribute to the gene-silencing effect of DNA methylation (Chao and Zoghbi 2009). In the absence of stimulation, MeCP2 occupies a site on the *Bdnf* promoter repressing the transcription of *Bdnf* (Martinowich et al. 2003). Neuronal depolarization dissociates MeCP2 from the *Bdnf* promoter resulting in its demethylation and *Bdnf* transcription (Chen et al. 2003). Gomez-Pinilla and coworkers (2011) showed that exercise stimulates DNA demethylation in *Bdnf* promoter IV, and elevates levels of activated MeCP2, as well as BDNF mRNA and protein in the rat hippocampus. The same study revealed that exercise increases acetylation of histone 3. Earlier, Tsankova and colleagues (2006) did show another epigenetic mechanism of BDNF regulation: exercise reduces levels of the histone deacetylase (HDAC) 5 mRNA and protein, implicated in the regulation of the *Bdnf* gene. Another study showed that inhibition of HDAC activity resulted in increased occupancy of the promoter I by acetylated histone 3, and increased levels of H3AcK9 and H3AcK14 proteins. In parallel HDAC inhibition caused upregulation of its mRNA and protein (Tian et al. 2010). It is plausible than, that similar mechanisms, remodeling chromatin containing the *Bdnf* gene, operate in the spinal cord.

Spinal cord neurons can adapt to increased activity of the circuitries involved in locomotion. Widenfalk and others (1999) reported that in spontaneously hypertensive rats, which are known to voluntarily run up to 20 km/night, long-distance running, does not evoke any robust neurotrophin changes in the spinal cord.

What is the spinal map of cells receptive to BDNF and NT-4 stimuli, modeled by $TrkB^{FL}$ receptor distribution pattern?

High levels of TrkB mRNA found in large neurons of motor nuclei (Piehl et al. 1994, Copray and Kernell 2000, Macias et al. 2007) and in several classes of smaller cells distributed in laminae VII–VIII (Macias

et al. 2007) identify multiple targets of BDNF/NT-4 signaling. The presence of TrkB transcripts in the nucleus intermedialis and in the lateral spinal nucleus neurons indicates that also sympathetic and sensory visceral centers are under control of BDNF/NT-4 and that locomotor training and other stimuli affecting spinal BDNF content may regulate their function. The receptor protein is present both in neuronal and glial perikarya and in neuronal and glial fibers (Skup et al. 2002, Gomez-Pinilla et al. 2004, Macias et al. 2005, 2007, Ziemlińska et al. 2014).

Importantly for the treatments/strategies aimed to increase BDNF and/or NT-4 signaling, BDNF gene transfer to the transected spinal cord, which caused long-term BDNF transgene overexpression, leading to over 100-fold increase of BDNF protein above control levels (Ziemlińska et al. 2014), reduced but did not abolish TrkB signaling (Skup et al. 2011) (Ziemlińska et al. in preparation) and was accompanied by spectacular stepping improvement in spinal animals (Boyce et al. 2012, Ziemlińska et al. 2014). More physiological, moderate BDNF increase shown to occur after long-term locomotor training (four weeks), does not change significantly an overall TrkB mRNA expression but is accompanied by an increase of the number of TrkB mRNA expressing cells, including astro- and oligodendrocytes (Macias et al. 2007). More precisely, in these conditions in many neurons in the lumbar ventral horn which demonstrate an increase of BDNF mRNA level there is also an increase of TrkB mRNA level (Macias et al. 2007). Importantly, not only long-term but also short-term (one week) training is effective in an enhancement of $TrkB^{FL}$ protein level in non-neuronal, mostly oligodendroglial cells in the spinal cord (Macias et al. 2005). The latter process likely manifests/reflects the recruitment of neurotrophin- non responding ("silent") cell pool to the population which is regulated by BDNF/ NT4 signals and may be indicative of increased involvement of glial cells in plasticity of the spinal network, indispensable in control and myelination of rearranging fibers (Tolwani et al. 2004, Xiao et al. 2010, Wong et al. 2013).

Therefore we may postulate that in the spinal cord there is a fast onset of the TrkB protein increase after training, leading to maintenance of this state afterwards, if exercise is extended. Moreover, based on subcellular distribution of $TrkB^{FL}$ protein in neuronal perikarya in the sedentary and exercised rats, which is predominantly cytoplasmatic in both functional states (Macias et al. 2005) we may speculate that exercise promotes the receptor pool to a dynamic exchange within the plasmalemmal compartment without its clear enrichment in $TrkB^{FL}$ protein, whereas an excess of the ligand reduces $TrkB^{FL}$ availability, a phenomenon which was observed predominantly in the fiber network (Ziemlińska et al. in preparation).

Complete spinal cord transection reduces BDNF availability in the network located caudally to injury

Until recently, a marked reduction of BDNF at the level of mRNA (by 40%) and much less at the protein level (15%), was found only if the spinal cord has been completely cut off from both supraspinal and peripheral inputs (Gomez-Pinilla et al. 2004). In other cases BDNF levels in neurons were reported to either increase, remain unchanged or moderately decrease after spinal cord transection calling into question a pronounced effect of BDNF impoverishment on dysfunction of spinal circuits. If BDNF did not change significantly, a tendency to increase TrkB mRNA at 10 days posttransection and a delayed increase of TrkB protein at one month, concomitant with increased NT-4 levels was noted (Keeler et al. 2012). These data rather support the view on a maintained contribution of BDNF- and NT-4 mediated signaling in modulation of the lumbar network in the transected spinal cord, at least within the first postoperative month. However, a recent detailed analysis of BDNF concentration and quantitation of long-term segmental changes in BDNF levels caused by a complete spinal transection in the rat, revealed a clear tendency of BDNF levels to decrease in the caudal lumbar segments at 7 weeks post-transection that was accompanied by a profound decrease in BDNF levels in the rostral lumbar segments (Ziemlińska et al. 2014), suggesting a progressing decline of BDNF levels (Gomez-Pinilla et al. 2004). Concomitantly with downregulation of TrkB mRNA and protein which occur at that post-transection period (Skup et al. 2011) these changes are indicative of long-term impairment of BDNF/TrkB signaling within the circuits of the rostral and, to a lesser extent, also of the caudal lumbar segments in the adult rat. An overall picture emerging is that both a decreased neurotrophic support and altered TrkB availability in target neurons contribute to long-term impairment of function of the transected spinal cord, and that the degree of dysfunc-

tion is dependent on the distance from the lesion along the rostro-caudal spinal axis. Strategies aimed to compensate for BDNF deficits to improve spinal functions were undertaken by several groups.

BDNF delivery and/or sensorimotor training augment the efficacy of spinal circuitries and improve stepping behavior after spinal cord transection

Both, BDNF delivery and/or sensorimotor training, which elevate spinal cord levels of BDNF, improve stepping behavior after spinal cord transection by augmenting the plasticity and efficacy of lumbo-sacral spinal networks. In adult rats with spinal cord injury, these treatments activate a large population of spinal neurons, as revealed with c-Fos mapping, normalize the levels of cyclic AMP response element binding protein (CREB) and synapsin 1, and result in axonal regrowth/sprouting and possibly sparing of severed axons and the density of synaptic inputs to lumbar motoneurons. Our recent study revealed that BDNF, when overexpressed caudally to transection site, in the networks isolated from descending inputs, up-regulates expression of the molecules involved directly in spinal neurotransmission (Ziemlińska et al. 2014). In these conditions, characterized by BDNF concentrations in the lumbar segments much above physiological range, the levels of markers of excitatory and inhibitory transmission, decreased by the lesion, returned to (L1–2 segments) or surpassed (L3–6) control levels in BDNF-treated rats. The same study revealed that increased expression of enzymes which control GABA content was accompanied by an increase of GAD67 in the terminals around motoneuronal perikarya. Therefore, the inhibitory inputs to motoneurons are strengthened. This is in agreement with results showing that BDNF treatment mainly support sprouting of F-type boutons with presumably inhibitory function (Novikov et al. 2000). Considering the functional consequences of these changes, it is noteworthy that abnormally high glutamate and glycine levels in the lumbar spinal cord in trained spinalized rats were found to correlate with the ability to perform independent stepping (Cantoria et al. 2011). We propose that in conditions of a permanent loss of the supraspinal control, locomotor stepping requires an elevation of both excitatory and inhibitory signaling from the remaining circuits above controls. Abnormally high levels of GABA accompanied by an elevated expression of VGluT2 mRNA, found in the caudal lumbar segments in spinal-BDNF-treated rats, support this reasoning (Ziemlińska et al. 2014). Noteworthy, in a parallel study with the same experimental paradigm, where L1 cell adhesion molecule transgene was applied to investigate its regenerative potential, no functional improvement was achieved (Płatek 2014). Comparison of the degree of recovery of neurotransmitter markers in both experiments showed, that albeit spinal rats overproducing L1 demonstrate a promising increase of GlyT2, GAD67 and VGluT2 mRNA levels towards controls, there is no increase above control values in any of these markers, contrary to what has been achieved in the BDNF- overproducing group (Fig. 3). These comparisons support our proposi-

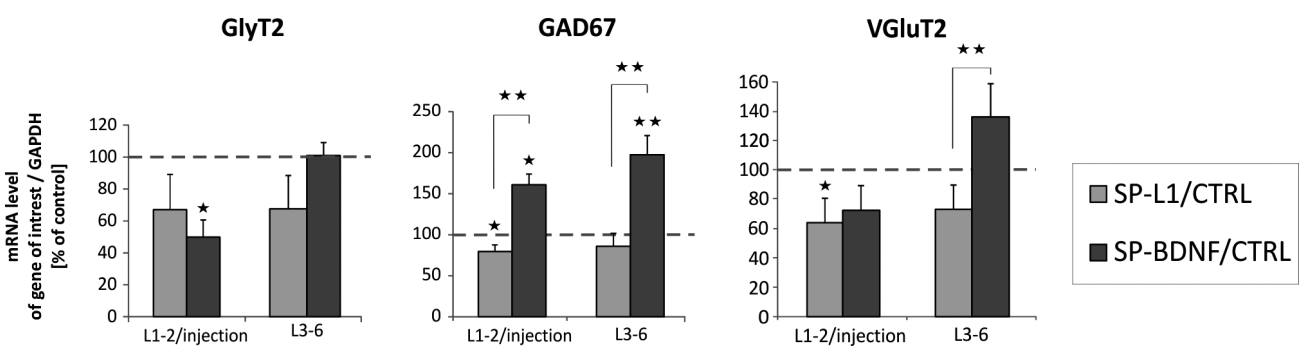

Fig. 3. Changes of GlyT2, GAD67 and VGluT2 transcripts in the lumbar segments of the spinal rats after injections of viral vectors AAV5-L1 (light grey) or AAV1/2-BDNF (dark grey) to the rostral lumbar (L1–2) segments. Transcripts levels are expressed as a percent of those in intact control rats (mean ± SD). AAV5-L1 (n=7), AAV1/2-BDNF (n=4), control groups (CTRL) n=4 and n=5, respectively. Transcripts levels were evaluated five weeks after spinalization and viral vector injections in AAV5-L1 group and two weeks later in AAV1/2-BDNF. Mann-Whitney U test was used for comparisons *P<0.05; **P<0.01 (From: Ziemlińska et al. 2014, Płatek 2014, with permission from Authors); GlyT2 data – Ziemlińska et al., unpublished).

tion on the conditions to be fulfilled to achieve the ability to generate alternating hindlimb stepping, when supraspinal control over the circuits of the lumbar segments is lost.

It has to be stressed that while the adequate strength of motoneuronal inputs is a prerequisite to generate stepping, the maintenance and precise regulation of motoneuron properties is equally important to achieve satisfactory recovery of locomotor function and avoid spasticity. Therefore treatments aimed to modify spinal inputs to motoneurons have to be considered in terms of their potential activity towards motoneurons as well. To this end, the effect of neurotrophins on expression, regulation, and distribution of membrane-bound ion channels, such as Kv2.1, large-conductance calcium gated potassium channels (BK) and small conductance (SK) channels, which contribute to the control of the intrinsic excitability of α-motoneurons (Rothberg, 2012) and undergo down-regulation by spinalization and training, is among the most important ones to evaluate (Muennich and Fyffe 2004, Deardorff et al. 2013). These issues await investigation, but limited data already show e.g. that increased BDNF contributes to reduced BK channel activity in DRG neurons (Cao et al. 2012), which would lead to increased excitability, while NT-3 but not BDNF exert an opposite effect on cortical neurons (Holm et al. 1997). We agree with the concept expressed recently by Boyce and coauthors (2012) that each neurotrophic factor has its own electrophysiological signature, which may be used to predict the changes in cell excitability and perhaps recovery of plantar stepping when it is expressed in the spinal cord after spinalization. Increased motoneuron susceptibility to discharge after BDNF delivery but not NT-3 delivery was a base of this concept (Boyce et al. 2012). Whereas the role of BDNF in control of neuron excitability and locomotion needs to be investigated further, together with acute administration of BDNF upregulating spinal levels of plasmalemmal KCC2 in spinal rats (Boulenguez et al. 2010), available data suggest that BDNF, depending on the dose- and time period of its elevation, alters the levels of presynaptic and postsynaptic systems caudal to the transection site. Our expectation that increased, sustained spinal levels of BDNF will lead to facilitation of KCC2 activity, did not come true (Ziemlińska et al. 2014). Strict temporal and spatial control of BDNF expression may help to maintain the appropriate level of excitability of the spinal networks providing optimal conditions to bring functional improvement.

CONCLUSIONS

To conclude, well documented effects of BDNF on dendritic and axonal growth, on expression of the proteins which contribute to neurotransmitter synthesis and release, and expression of those which regulate the ability of the neuron to respond to its inputs, suggest that an increase of neurotrophin signaling after spinal injury, if produced by locomotor training or electrical stimulation, may be a prerequisite for morphological rearrangement and neuron receptiveness also in humans.

There is a growing consensus that recovery largely depends on plasticity phenomena induced by the lesion (Calancie et al. 1994, Wernig et al. 1995, Harkema et al. 1997, Barbeau et al. 1999a,b, Dietz et al. 1999, Dobkin 2000, Raineteau and Schwab 2001, Calancie et al. 2002). Daily locomotor training with body-weight-support on a treadmill (BWST) often results in significant improvements in locomotor function in motor-incomplete SCI patients (Dietz et al. 1995, 1998, Dobkin et al. 1995, Wernig et al. 1995, Barbeau et al. 1999a,b, Barbeau and Fung 2001). The potential of motor circuits in the human spinal cord to rearrange after spinal lesion and locomotor training is preserved (Grasso et al. 2004). This study did show that, when the recorded patterns of muscle activity were mapped onto the approximate rostrocaudal location of motoneuron pools in the human spinal cord, the reconstructed spatiotemporal maps of motoneuron activity in SCI patients were quite different from those of healthy subjects. At the end of training, the locomotor network reorganized at both supralesional and sublesional levels indicating that locomotor responses in SCI patients may not be subserved by changes localized to limited regions of the spinal cord, but may depend on redistribution of activity across most of the rostrocaudal extent of the spinal cord. Three out of four available human studies revealed that (Ferris et al. 2007, Cho et al. 2012, Schmolesky et al. 2013) serum and platelet BDNF levels in humans are significantly elevated in response to exercise, and the magnitude of increase is exercise intensity dependent (but see also Goda et al. 2013). In light of these human studies and the data collected in animal models it is conceivable that BDNF signaling plays an important role in spinal function and plasticity of the locomotor circuits also in humans.

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