Experimental Neurology xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Anti-Nogo-A and training: Can one plus one equal three?

Michelle L. Starkey *, Martin E. Schwab

Brain Research Institute, University of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 8 November 2010
Revised 15 March 2011
Accepted 7 April 2011
Available online xxxx

Keywords:
Nogo-A
Rehabilitation
Training
Spinal cord injury
Central nervous system

ABSTRACT

Following spinal cord injury (SCI) the adult central nervous system (CNS) has a limited but substantial capacity for repair and plastic reorganisation. The degree of reorganisation is determined by a number of factors such as the extent and location of the lesion, the remaining circuit activity within the CNS and the age at injury. However, even in the best cases this spontaneous reorganisation does not lead to full recovery of the affected behaviour but instead often results in a functionally successful but compensatory strategy. Current SCI research focuses on enhancing fibre tract (re-)growth and recovery processes. Two currently promising approaches are the neutralisation of CNS growth inhibitory factors, and rehabilitative training of remaining networks. Independently, both approaches can lead to substantial functional recovery and anatomical reorganisation. In this review we focus on Nogo-A, a neurite growth inhibitory protein present in the adult CNS, and its role in regenerative and plastic growth following SCI. We then discuss the efforts of rehabilitative training and the potential combination of the two therapies.

Introduction

Despite a growing list of potential future treatments for SCI, there is still a great need to precisely understand the underlying mechanisms. This is especially important when thinking about combinatorial approaches to aid recovery from SCI. It is hoped that an understanding, from the cell biological point of view, of the complex physiological and pathophysiological processes following SCI, rehabilitation, training and regeneration enhancing pharmacological treatments will allow an insight into how to optimally combine these manipulations to maximise functional recovery. In this review we focus first on the spontaneous adaptive changes that occur in the spinal cord and brain circuitry following SCI. Next, we summarise two strategies that can enhance this reorganisation and thus lead to extensive functional recovery: neutralising the growth inhibitory protein Nogo-A and rehabilitative training. Blocking the function of Nogo-A following SCI allows regenerative growth of damaged axons and promotes plastic sprouting of intact fibres along with improved functional recovery. Rehabilitative training strategies are widely used experimentally and in the clinic. Recent findings with combinations of growth enhancing interventions and various training paradigms produced unexpectedly complex outcomes. These results point to a great need for a better understanding of the underlying mechanisms in order to best optimise future therapeutic strategies.

Processes of spontaneous recovery after SCI

The adult CNS has a limited but substantial capacity for spontaneous plastic reorganisation after injury (Merzenich et al., 1983b; Bareyre et al., 2004; Frigon and Rossignol, 2006; Edgerton et al., 2008; Darian-Smith, 2009; Ghosh et al., 2010; Ramer, 2010; Rosenzweig et al., 2010). Long distance regeneration of injured neurons does not occur spontaneously, but spared fibres, and surviving circuits in the brain and lower spinal cord can sprout, reorganise and contribute to functional recovery and compensation.

Spontaneous reorganisation of the sensory and motor forebrain cortex following injury

The forebrain cortex is capable of spontaneous reorganisation following injury in adulthood to peripheral nerves or the spinal cord. The majority of early data came from peripheral nerve lesion experiments in primates (Merzenich et al., 1983a; Merzenich et al., 1983b; Donoghue et al., 1990; Kaas, 1991): areas of the cortex corresponding to the lesioned nerve initially became silenced, but were rapidly "filled in" by responses from surrounding, intact areas (Merzenich et al., 1983a; Merzenich et al., 1983b; Sanes et al., 1988). Similar reorganisation was observed in the adult rat sensory and motor cortex following SCI (Fig. 1B1); following partial SCI in adult rodents neighbouring, intact areas of the cortex spontaneously expanded into the regions that had lost their targets, as observed with intracortical microstimulation (Fouad et al., 2001; Martinez et al., 2009), electrophysiology (Aguilar et al., 2010), trans-synaptic tracing (Bareyre et al., 2004), functional magnetic resonance imaging (fMRI) (Endo et al., 2007; Ghosh et al., 2009; Nishimura and Isa, 2009; Ghosh

* Corresponding author.
E-mail address: starkey@hifo.uzh.ch (M.L. Starkey).

0014-4886/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2011.04.008

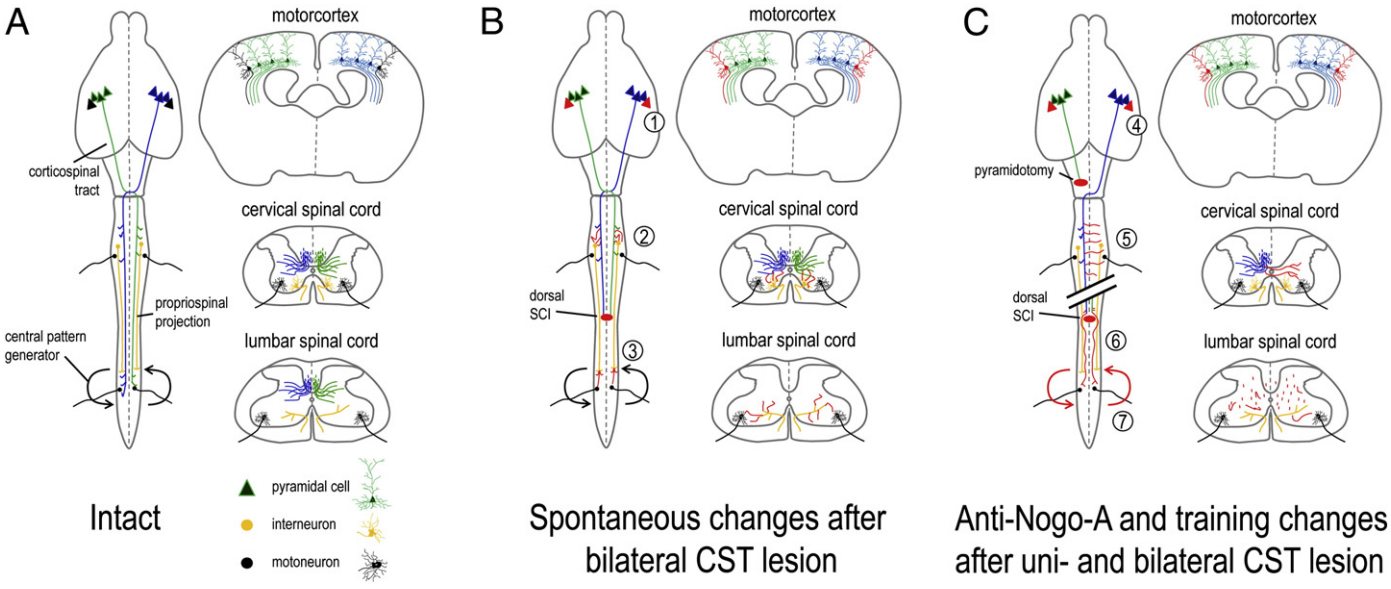

Fig. 1. Anti-Nogo-A treatment and rehabilitative training induce anatomical changes post-SCI. **A**, Intact adult CNS of a rat. Layer V pyramidal cells (green and blue) composing the CST send crossed projections from the motor cortex to the spinal cord. Axons from the pyramidal cells terminate in the cervical (from the forelimb motor cortex) and lumbar (from the hindlimb motor cortex) spinal cord grey matter. For illustrative purposes, two spinal cell types are shown. Long descending propriospinal interneurons (yellow) project from the cervical to the lumbar spinal cord. Motoneurons (black) are typically located in the ventral horn of the cervical and lumbar spinal cord and innervate the muscles of fore- and hindlimbs. Circular arrows indicate the spinal central pattern generator networks. **B**, Spontaneous changes occurring after bilateral CST lesion in the adult rat. In the absence of long-distance regeneration considerable functional recovery occurs spontaneously, mediated by: 1) undamaged motor cortex cells (red pyramidal cells) taking over the function of damaged neurons; 2) injured fibres disconnected from their original target in the lumbar spinal cord sprouting into the cervical spinal cord (red sprouts) and connecting onto local long propriospinal neurons (yellow), which bridge the lesion site; and 3) these propriospinal neurons increasing their terminal arborisation (red sprouts) in the lumbar spinal cord. **C**, Effects of anti-Nogo-A treatment and rehabilitative training following unilateral (illustrated in the upper part of the cartoon) and bilateral (illustrated in the lower part of the cartoon) CST lesion: 4) anti-Nogo-A treatment cells allows sprouting and enhanced growth of cortical neurons (red pyramidal cells); training allows growth of dendritic spines; 5) following unilateral CST lesion, both removal of Nogo-A and enhanced activity induce bilateral innervation (red fibres) of the cervical spinal cord by intact fibres; 6) after SCI, anti-Nogo-A treatment produces increased sprouting of lesioned fibres rostral to the lesion site and long-distance regeneration of injured axons (red fibres), 7) rehabilitative training induces adaptations in central pattern generator networks (red arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2010), voltage-sensitive dye imaging (VSD) (Ghosh et al., 2009; Ghosh et al., 2010) and retrograde tracing (Ghosh et al., 2010). Cortical plasticity following partial SCI has also been observed in humans with positron emission tomography (Bruehlmeier et al., 1998; Curt et al., 2002b) and fMRI (Curt et al., 2002a); for review see (Endo et al., 2009). In addition, it was shown in monkeys that recovery of hand function following partial cervical SCI was accompanied by the re-emergence of the cortical map of the hand (Schmidlin et al., 2004). The above observations suggest that the detailed structure of cortical maps is dynamically maintained throughout life, and that maps are able to rapidly respond to changing inputs. However, the exact mechanism(s) underlying these changes are not known but may involve long-term potentiation (Hess and Donoghue, 1994; Rioult-Pedotti et al., 2007), unmasking of horizontal connections (Hess and Donoghue, 1996; Adesnik and Scanziani, 2010), removal of inhibition (Hendry and Jones, 1986; Jacobs and Donoghue, 1991; Brasil-Neto et al., 1993; Aguilar et al., 2010), and/or sprouting and growth of new connections (Kleim et al., 2004; Kim et al., 2008; Ghosh et al., 2010).

Spontaneous reorganisation of spinal connections following injury

While functional and anatomical plasticity in the neocortex are well studied, recent observations suggest that the spinal cord is capable of substantial plastic changes also. Three fibre systems have been studied in detail: the hindlimb corticospinal tract (CST) (Fig. 1B2) (Fouad et al., 2001; Ghosh et al., 2009; Ghosh et al., 2010), propriospinal connections (Fig. 1B2 & B3) (Bareyre et al., 2004; Courtine et al., 2008) and fibre networks including central pattern generators (Lovely et al., 1986; Rossignol et al., 2002; Edgerton et al., 2004; Barriere et al., 2008; Edgerton et al., 2008; Ichiyama et al., 2011). Four weeks after mid-thoracic transection in the adult rat, stimulation of the hindlimb somatosensory cortex, which before the lesion yielded only hindlimb movements, produced forelimb, trunk and shoulder responses (Fouad et al., 2001). This functional reorganisation of the cortex was associated with extensive sprouting of the lesioned hindlimb CST fibres into the cervical spinal cord (Fouad et al., 2001; Ghosh et al., 2009; Ghosh et al., 2010). fMRI and VSD imaging showed that the forelimb sensory representation had also expanded into the hindlimb area (Ghosh et al., 2010). Whereas many of the former hindlimb fibres now assumed forelimb functions, retrograde tracing and electrophysiology showed that some of the sprouts of hindlimb axons in the cervical spinal cord connected to long propriospinal neurons which bridged the damaged area and thus served as a relay reconnecting the intact cervical spinal cord to the denervated lumbar spinal cord (Bareyre et al., 2004; Kerschensteiner et al., 2004). In an extension of these findings it was shown that these newly formed propriospinal relay connections, which bypassed the lesion site, were able to mediate recovery of treadmill locomotion (Courtine et al., 2008). It is known that functional and structural changes also occur in the spinal networks caudal to the lesion following SCI, for example in the spinal circuits that generate motor patterns for walking. Such changes have been extensively studied in animals and humans (Cai et al., 2006; Frigon and Rossignol, 2006; Edgerton et al., 2008; Courtine et al., 2009; Dietz, 2010). A detailed overview can be found in other reviews in this edition, and so will not be discussed further here.

Factors affecting spontaneous recovery

A number of factors affect spontaneous recovery after SCI. Whereas both the size (Burns et al., 1997; Little et al., 1999; Schucht et al., 2002; Multon et al., 2003; Piecharka et al., 2005; Hsu and Jones,

Please cite this article as: Starkey, M.L., Schwab, M.E., Anti-Nogo-A and training: Can one plus one equal three? Exp. Neurol. (2011), doi:10.1016/j.expneurol.2011.04.008

2006; Spiess et al., 2009) and the rostrocaudal, ventrolateral location (Fouad et al., 2000; Schucht et al., 2002; Dietz and Colombo, 2004; Kanagal and Muir, 2009) of the lesion play a key role, in this review we will focus on two other important factors. Firstly, the age of the animal/patient at the time of injury can have a significant effect on the degree of spontaneous recovery. In the postnatal period, progressive myelination in the CNS leads to mounting levels of factors that are inhibitory for neurite growth. Thus, with increasing age the likelihood of spontaneous fibre growth tends to decrease (Wang et al., 2004; Bilston and Brown, 2007; Takahashi et al., 2009; Choi et al., 2010; Tillakaratne et al., 2010). In line with this, when myelin was experimentally removed from the spinal cord, regeneration of injured CST fibres was enhanced (Savio and Schwab, 1990) and the expression of a growth-associated protein (GAP-43) increased (Kapfhammer and Schwab, 1994). The second factor that has an impact on the degree of spontaneous recovery is the amount of rehabilitative "self- training" by the animal/patient. Older animals/humans tend to be less active than younger ones (van Hedel and Dietz, 2004; Harmsen et al., 2006; Holme et al., 2007), and translating the positive effects of rehabilitative training can become difficult with increasing age (Jakob et al., 2009). The amount of self-training/activity is also greatly influenced by housing/hospital conditions (Lankhorst et al., 2001; Wurbel, 2001; Teasell et al., 2005; Dai et al., 2009) as discussed below.

Blocking Nogo-A: an approach for enhancing recovery

One reason for the limited spontaneous anatomical and functional recovery in the adult CNS post-lesion is the presence of negative regulators of neurite outgrowth (Schwab, 2004; Yiu and He, 2006; Schwab, 2010). In particular CNS myelin is a highly non-permissive substrate for growth and, up to now, about ten different proteins with neurite growth inhibitory activity *in vitro* have been found in CNS myelin (Yiu and He, 2006; Schwab, 2010). One of the most potent and best studied of these growth inhibitors is Nogo-A; its neutralisation or removal from CNS myelin yields a growth-permissive substrate (Caroni and Schwab, 1988; Chen et al., 2000; Oertle et al., 2003). Addition of neutralising antibodies to spinal cord sections *in vitro* converted CNS white matter into a permissive substrate on which cells were able to adhere and elongate (Savio and Schwab, 1989) and infusion into spinal cord lesioned animals allowed growth and plastic sprouting of damaged and intact fibres, respectively (Schnell and Schwab, 1990; Liebscher et al., 2005; Freund et al., 2007; Mullner et al., 2008; Maier et al., 2009; Gonzenbach et al., 2010). Recently, a human version of an anti-Nogo-A antibody (ATI355) has been produced and is currently under clinical trial.

Nogo-A is the longest splice form generated by the *nogo* gene (Chen et al., 2000; GrandPre et al., 2000; Oertle et al., 2003). All three isoforms of *nogo* mRNAs (Nogo-A, -B and -C) share a common C-terminal domain, the reticulon-homology domain, hence their inclusion in the reticulon gene family (GrandPre et al., 2000; Oertle and Schwab, 2003). The inhibitory nature of Nogo-A is attributed to two extracellular regions of the protein: the C-terminal Nogo-66 domain and a sequence referred to as Nogo-Δ20 or amino-Nogo (GrandPre et al., 2000; Fournier et al., 2002; Oertle et al., 2003). The Nogo-66 domain binds to the Nogo-66-receptor (NgR1) (Fournier et al., 2001; Fournier et al., 2002), as do two other myelin-associated neurite growth inhibitors, myelin-associated glycoprotein MAG (McKerracher et al., 1994) and oligodendrocyte myelin glycoprotein OMgp (Domeniconi et al., 2002; Liu et al., 2002; Wang et al., 2002b; Venkatesh et al., 2007). NgR1 does not have a transmembrane domain and so it must be associated with transmembrane proteins for signalling to occur; Lingo-1, TROY, and p75 were found so far (Wang et al., 2002a; Mi et al., 2004; Park et al., 2005). The Nogo-66 domain also binds to PirB (paired immunoglobulin-like receptor B) and blocking of PirB or NgR1 can overcome myelin inhibition (Atwal et al., 2008). High affinity binding of Nogo-Δ20 has been shown biochemically (Oertle et al., 2003) but the binding partner/receptor is not yet fully characterised. Subsequent to binding, it is known that Nogo-Δ20 is internalised into neuronal cells where it activates RhoA and the rho effector, ROCK (Joset et al., 2010). LIM kinase and the actin-regulating protein Cofilin are involved in transmitting signals to the axonal cytoskeleton (Montani et al., 2009; Nash et al., 2009; Schwab, 2010). Although other CNS myelin proteins that inhibit neurite growth were found, e.g., MAG, OMgp, semaphorin 4D (Moreau-Fauvarque et al., 2003), semaphoring 5A (Goldberg et al., 2004), ephrin B3 (Benson et al., 2005), netrin (Low et al., 2008) and certain proteoglycans (Yiu and He, 2006), Nogo-A remains the most restrictive inhibitory molecule currently known and plays an important role in the inhibition of sprouting and regenerative neurite growth in the CNS (Cafferty et al., 2010; Schwab, 2010).

Neutralisation of Nogo-A enhances regeneration, growth and recovery following injury

In the early 1990s, a significant step forward in the field of SCI occurred when the regeneration of injured CNS axons in rats treated with an anti-Nogo-A antibody (IN-1) was described (Schnell and Schwab, 1990). Large, dorsal, mid-thoracic SCIs in adult rats produced a sprouting response in lesioned fibres rostral to and at the lesion site. However, only in those animals receiving anti-Nogo-A antibodies were these fibres seen to grow around the injury site and into the caudal spinal cord (Fig. 1C6) (Schnell and Schwab, 1990; Liebscher et al., 2005). This fibre growth was associated with significant functional recovery of CST-mediated behaviours (Bregman et al., 1995) and of locomotor ability; over-ground walking, treadmill locomotion, narrow beam crossing and swimming (Merkler et al., 2001; Liebscher et al., 2005). Lesioned rats treated with an anti-Nogo-A antibody had alternating EMG (electromyogram) recordings for flexor and extensor muscles when walking on a treadmill, whereas lesioned animals treated with a control antibody displayed only minimal rhythmic activity (Merkler et al., 2001). Using fMRI it was shown that anti-Nogo-A antibody treated lesioned animals had a reactivation of the hindlimb sensory cortex which had become silenced by the lesion (Fig. 1C4), (Liebscher et al., 2005). Importantly, removal of Nogo-A has never been associated with increased pain (hyperalgesia) (Merkler et al., 2001; Maier et al., 2009; Willi et al., 2009).

Many of these findings have been reproduced in other laboratories using different CNS lesions, treatment paradigms, treatment types (antibodies, the NgR1 blocking peptide NEP1–40, soluble NgR-Fc fusion proteins) and in various species including primates (Freund et al., 2006; Freund et al., 2007; Freund et al., 2009). For example, following a mid-cervical unilateral hemisection in adult macaque monkeys, manual dexterity recovered significantly better in the monkeys treated with anti-Nogo-A antibody than in controls (Freund et al., 2006, 2009). Antibody-treated monkeys had a greater number of CST fibres caudal to the lesion (Freund et al., 2006), a greater number of sprouting axons and midline crossing fibres, and fewer retraction bulbs rostral to the lesion (Freund et al., 2007). In rodents, blockade of NgR1 with the antagonist NEP1-40 also promoted CST growth and functional recovery *in vivo* (GrandPre et al., 2002), even when the treatment was delayed or given systemically (Li and Strittmatter, 2003). Administration of a function-blocking soluble NgR1 protein fragment after SCI in adult rats also lead to axonal growth and electrophysiological and functional recovery (Li et al., 2004), and blocking Lingo-1 in rats after SCI improved functional recovery and increased axonal sprouting (Ji et al., 2006).

However, the results from studies knocking out the *nogo-A* gene (and also other Nogo isoforms) were initially less convincing. The controversy began when two different laboratories both reported enhanced sprouting and axonal regeneration following SCI in two separate knockout lines (one lacking Nogo-A and the other Nogo-A and -B) (Kim et al., 2003; Simonen et al., 2003), whereas a third

Please cite this article as: Starkey, M.L., Schwab, M.E., Anti-Nogo-A and training: Can one plus one equal three? Exp. Neurol. (2011), doi:10.1016/j.expneurol.2011.04.008

laboratory saw no significant enhancement of recovery in either a Nogo-A/B or Nogo-A/B/C knockout line (Zheng et al., 2003). Not surprisingly, the background strain of the mice (Dimou et al., 2006) and compensatory up-regulation of other growth inhibitory proteins were shown to play important roles (Schwab, 2010). Moving away from SCI, enhanced plastic sprouting and functional recovery was present in all strains following unilateral pyramidotomy (transection of the CST at the level of the pyramids in the brainstem) (Cafferty and Strittmatter, 2006), and plastic reorganisation of intact fibres and functional recovery was observed following unilateral stroke in mice lacking either NgR1 or Nogo-A/B (Lee et al., 2004). Following SCI in triple knockouts (of Nogo-A, MAG, and OMgp) enhanced sprouting was reported to be mainly due to the removal of Nogo-A (Cafferty et al., 2010; Lee et al., 2010). However, in this case, enhanced axonal re-growth (CST and raphespinal) and improved locomotion was seen in one laboratory/mouse line (Cafferty et al., 2010) but not in another (Lee et al., 2010).

It should be noted that in the many regeneration studies reported, the number of fibres actually showing long distance regeneration is only modest. However, as mentioned above, as long as some intact tissue remains, enhanced sprouting of unlesioned fibres can also contribute to recovery. Anti-Nogo-A treatments have also been shown to enhance sprouting and growth of intact fibres. This was most clearly shown after unilateral pyramidotomy or stroke both of which leave one CST projection to the spinal cord intact: anti-Nogo-A antibody administered post-lesion lead to sprouting of the intact CST across the spinal cord midline leading to bilateral innervation of the cervical spinal cord, red nucleus and pons by the intact CST (Fig. 1C5) (Thallmair et al., 1998; Z'Graggen et al., 1998; Blochlinger et al., 2001; Papadopoulos et al., 2002; Cafferty and Strittmatter, 2006). This novel innervation was associated with recovery of precise forelimb movements and sensation in the impaired body parts (Thallmair et al., 1998; Papadopoulos et al., 2002).

Plastic and regenerative events mediated by Nogo-A-suppression are not restricted to the CST. Sprouting of the intact rubrospinal tract into the ventral horn of the spinal cord was observed following bilateral pyramidotomy and anti-Nogo-A treatment (Raineteau et al., 2002). Also mice lacking NgR1 showed regeneration of raphe- and rubrospinal fibres following SCI which was associated with significant functional recovery (Kim et al., 2004). Serotonergic fibres were also able to re-establish lamina-specific connections in the lumbar spinal cord following SCI and either anti-Nogo-A (Mullner et al., 2008) or NEP1-40 treatment (Li and Strittmatter, 2003). Anti-Nogo-A treatment of the adult CNS also enhanced growth and plasticity in the absence of an injury. Intact purkinje cells of the cerebellum reacted to a single injection of anti-Nogo-A antibody by up-regulating the expression of transcription factors and immediate early genes important for growth (Zagrebelsky et al., 1998) and by profuse axonal sprouting (Buffo et al., 2000). In addition, anti-Nogo-A treatment of intact, naive rats enhanced sprouting of CST fibres without affecting the gross behaviour of the animals (Bareyre et al., 2002). Dendritic sprouting and remodelling was also observed in the intact adult hippocampus after anti-Nogo-A antibody treatment, or knockout of either Nogo-A or NgR1 (Zagrebelsky et al., 2010). Finally, Nogo-A and NgR1 play a negative role in activity-dependent synaptic plasticity in the intact adult hippocampus (Raiker et al., 2010; Delekate et al., 2011).

Rehabilitative training to enhance recovery after spinal cord injury

A number of experimental studies suggest that training of specific functions after a CNS injury has positive effects on recovery. Clinically, the most successful therapy for SCI, stroke and brain injury is rehabilitative training. As other reviews in this edition focus on this type of therapy, here we discuss the important factors to consider when designing a combinatorial therapy involving rehabilitative training.

Task-specificity of training: be careful what you train?

When designing a rehabilitation programme it is important that training results in the recovery of functions that are useful in the everyday life of the animal or human. Whether training of one task can be converted to recovery of function in another, untrained task seems to depend on a number of factors including the type of training, the untrained task tested, the volume of training and the lesion. The expectation would be that as long as there is an overlap in the neural strategy used for the two movements (the trained and the untrained) then it should be possible to translate the training of one task to ability in another (Singh et al., 2010). The current experimental literature, despite covering many different training strategies, control groups, lesions and species, remains rather controversial.

Evidence against transfer of skills

Initial insights into the issue of converting training in one task to ability in another came with the intriguing finding that spinalised cats trained to stand stood well but did not step, whereas cats trained to step stepped well but could not stand (Edgerton et al., 1997). Intriguingly, if step trained cats were subsequently trained to stand they lost the ability to step (De Leon et al., 1998a, b). Also in humans, the ability to step forwards on a treadmill following SCI did not transfer to backwards stepping (Grasso et al., 2004). In another study, it seemed that ability in one task came at the expense of another: rats with an upper cervical lesion were trained post-lesion to grasp. Whereas these rats showed recovery in this task their ability to cross a horizontal ladder was worse than that of untrained animals (Girgis et al., 2007; Krajacic et al., 2010). When grasping and environmental enrichment were used separately as training after dorsal column crush injury, environmental enrichment (for 1 hour per day) resulted in a reduced grasping ability (Garcia-Alias et al., 2009). However, environmental enrichment in combination with grasping and horizontal ladder training allowed recovery on both trained tasks but not of either over-ground locomotion or forelimb use in the cylinder test (Dai et al., 2009). Also, in rats with incomplete SCI swimming or wading training significantly improved the trained task but did not improve over-ground locomotion (Smith et al., 2006; Kuerzi et al., 2010).

Evidence for transfer of skills

Conversely, a number of studies have shown a transfer of training in one test paradigm to improved function in another. Treadmill training or wheel running early after SCI produced a quicker recovery of over-ground walking compared to controls (Multon et al., 2003; Engesser-Cesar et al., 2007). However, it should be noted that of course, both wheel running and treadmill training are very similar to over-ground locomotion. In another study, following mid-thoracic lesion both treadmill training and self-training were translated to enhanced recovery in a related but novel task, i.e., staircase stepping (Maier et al., 2009). When this task was used as a training paradigm following mid-thoracic contusion, rats improved significantly on the trained task (staircase stepping) and also showed recovery on the horizontal ladder and in detailed aspects of locomotion (CatWalk), although some abnormalities remained (Singh et al., 2010). These results suggest that rehabilitative therapies should train different tasks together as it seems that a lack of practise in one task can lead to it being lost or becoming non-functional.

Amount of training

In addition to the type of training the amount received is an important factor to consider. Although step training was shown to produce a quicker recovery of locomotor ability following complete

Please cite this article as: Starkey, M.L., Schwab, M.E., Anti-Nogo-A and training: Can one plus one equal three? Exp. Neurol. (2011), doi:10.1016/j.expneurol.2011.04.008

transection, untrained cats and rats also showed a high degree of spontaneous recovery (de Leon et al., 1998b), raising the question of how much training is required to have a major beneficial effect over what occurs spontaneously and by self-training (Fouad et al., 2000). Unsurprisingly, animals that made more steps (1000) in a treadmill session improved better than those making fewer (100) (Cha et al., 2007), and daily locomotor training lead to greater effects than only 3 sessions per week (Engesser-Cesar et al., 2007). As mentioned above, the amount of spontaneous self-training depends on the type and size of the injury and the resulting impairment and also on whether animals are housed in (active) groups or in isolation. If extensive self-training occurs it can allow recovery to a level that cannot be further improved by training (Kuerzi et al., 2010), the so called "ceiling effect". Standard protocols for treadmill training allocate only a small percentage of the day to actual training; rats or cats are usually trained for 20-30 min, 3–5 times per week and humans often 0.5–1 h, 1–5 times per week. However, it remains largely unknown if much longer and more frequent training sessions would improve functional recovery in tasks like locomotion or hand/forepaw use. In addition, activities in the non-training period might have an impact, either positive or negative, on the activities practised in the short training period and should be taken into account when interpreting the data.

Potential mechanisms underlying training effects

The current mechanistic understanding of rehabilitation is at best fragmentary but is important when planning combination treatments such as with anti-Nogo-A therapy. Refinement of newly established pathways following growth/plasticity enhancing treatments may depend on activity-dependent mechanisms. It is known that learning a skill is associated with increased dendritic complexity and an expansion of motor maps in areas involved in the learnt task (Adkins et al., 2006; Xu et al., 2009). Also, during development changes in neuronal activity shape the structure of connections, e.g., of the CST: inhibition of CST activity during the early post-natal period in cats leads to a defect in pruning of inappropriate connections (Martin et al., 1999). When use of a forelimb was blocked during development, topology and morphology of CST axon terminals were affected, and this was correlated with functional deficits that did not recover with age (Martin et al., 2004). Restriction of forelimb activity also reduced the forelimb representation in the motor cortex, but this enlarged again once activity returned (Martin et al., 2005). In turn, when CST axons or neurons were stimulated electrically during their refinement period, they occupied larger target territories than non-stimulated fibres (Salimi and Martin, 2004). Following adult unilateral pyramidotomy, chronic stimulation of the contralateral, intact CST axons or motor cortex enhanced sprouting of these fibres in the spinal cord, in particular by inducing growth over the midline and re-innervation of the denervated hemicord (Brus-Ramer et al., 2007; Carmel et al., 2010). In a similar way, forced-use of the impaired paw by constraint-induced movement therapy (applying a unilateral cast on the intact forelimb) following unilaterally pyramidotomy in adult rats increased branching of midline crossing CST fibres in the grey matter and enhanced synapse formation (Maier et al., 2008). These results suggest that increased neuronal activity can trigger or enhance compensatory growth of axonal branches and the formation of new connections. Encouragingly, as mentioned above, similar CST sprouting across the spinal cord midline was found after Nogo-A or NgR1 suppression (Thallmair et al., 1998; Lee et al., 2004; Cafferty and Strittmatter, 2006).

Combination of Anti-Nogo-A treatment and training to enhance recovery

With the research mentioned above as a background a small number of *in vivo* studies have now been carried out combining anti-Nogo-A therapies and rehabilitative training paradigms. How rehabilitative training should or could be combined with pharmacological interventions such as Nogo-A suppression is a question of crucial importance. One might expect that a more plastic CNS would be an advantageous background on which to apply rehabilitation as newly grown fibres are likely to need activity to stabilise or prune functional or non-functional connections, respectively.

In a first study (Maier et al., 2009) adult rats with an incomplete thoracic SCI received anti-Nogo-A antibody treatment and, simultaneously, daily forced treadmill training. Antibody alone (although probably with some spontaneous self-training) was associated with good recovery of locomotion with coordinated stepping, few paw drags, and a low variability in the step cycle. Rats that received treadmill training alone (and no antibody treatment) were well coordinated but dragged their paws and developed an abnormal low, long, sweeping gait. Surprisingly however, when the two treatments were given simultaneously the functional outcome was clearly worse than after either of the treatments alone. In terms of anatomy, all groups that received anti-Nogo-A antibody showed regeneration of CST and serotonin fibres, whereas those receiving training alone did not. The combination of training with anti-Nogo-A antibody treatment did not increase this growth over control levels (but importantly it also did not hinder it). These data seem to suggest that some sort of interference occurred between the mechanisms induced by the two treatments. The reason for the incompatibility between the two treatments is the subject of current research; one suggestion is that it might be due to different learning strategies — forced treadmill training vs. anti-Nogo-A facilitated self-training. It is also conceivable that a very plastic nervous system with multiple fibres and pathways sprouting/growing does not react positively to intense, forced training.

An important additional aspect is timing; in the above study the antibody treatment and training were given simultaneously, immediately after the lesion (Maier et al., 2009). In an extension of this work it was shown that delaying the treadmill training until two weeks after the end of the antibody treatment, i.e., four weeks after the lesion, lead to a very good functional outcome (Marsh et al., 2010). Also, following cervical hemisection in NgR1 knockout mice delayed (11–12 days post-injury) rehabilitative locomotor training, regardless of the genotype, lead to functional recovery in tasks related to the training (Harel et al., 2010). On the other hand, mice lacking NgR1 performed better on tasks unrelated to the training, independent of training but a synergistic effect was not reported. Conceptually, the most likely hypothesis is that training-induced selection and stabilisation of connections may have to follow an initial growth phase which is induced or enhanced by Nogo-A suppression or NgR1 removal.

In contrast to the results mentioned above, using a stroke paradigm, a recent study showed that when NgR1 was blocked whilst the animals carried out training for forelimb grasping, recovery of grasping occurred sooner and to a greater degree than when either of the treatments were applied alone (Fang et al., 2010). Why exactly there are differences between these studies is not yet clear. The effects of combining another plasticity-enhancing compound, Chondroitinase ABC (ChABC), with training have also been investigated (Garcia-Alias et al., 2009) and are the subject of another review in this edition.

As rehabilitation is an obligatory step in the current care protocols for SCI patients, any future novel therapy will have to work in combination with it (Dobkin and Havton, 2004; Dobrossy and Dunnett, 2005; Kim et al., 2008) and thus more experimental studies in this area are required. In human rehabilitation important factors to consider will be the timing, the type and the intensity of training, the motivational state of the patient, whether the training is forced or voluntary, and the (difficult to assess) degree of self-training. When investigating the complex interactions of therapeutic interventions, the functional readouts used will also be an important factor to

Please cite this article as: Starkey, M.L., Schwab, M.E., Anti-Nogo-A and training: Can one plus one equal three? Exp. Neurol. (2011), doi:10.1016/j.expneurol.2011.04.008

consider (Alexander et al., 2009; Zorner et al., 2010). Currently, a clinical trial of a human function blocking anti-Nogo-A antibody (ATI355) is underway in collaboration with Novartis (http://clinicaltrials.gov/ct2/show/NCT00406016). Phase I, which primarily investigates safety, dosing, tolerance and pharmacokinetics, started in 2006 and is now coming to a close with more than 50 acutely injured para- and tetraplegic patients. So far, no deleterious side effects of the treatment have been observed. In the planned Phase II of the trial treatment will start a few days after the injury, which corresponds to the initial spinal shock phase where intensive rehabilitation is not possible, and will be given over a few weeks. All patients will then receive the standard optimised rehabilitative treatment. Their outcomes will be compared to a large database of historical controls as well as to placebo treatment.

As will have become clear from this review, preclinical observations cannot currently recommend the best protocol for combining rehabilitative training with growth-enhancing treatments, e.g., anti-Nogo-A or Nogo receptor reagents or ChABC. The mechanisms behind functional recovery and repair of the structure of the CNS after an injury are just beginning to be unravelled. Clinical findings, which were once mostly empirical, are now starting to be understood on a basic science level; the insights gained are expected to help to improve our current treatment paradigms.

Acknowledgments

We would especially like to thank our colleague Björn Zörner for critical review of and discussion about the manuscript, and for help in preparing the figure. Also special thanks to Christiane Bleul for help and support in preparing the manuscript, and Linard Filli for comments on the final draft. The authors' lab is supported by grants of the Swiss National Science Foundation (grant 3100AO-122527/1), the National Centre for Competence in Research "Neural Plasticity & Repair" of the Swiss National Science Foundation, The European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreements no 201024 and no 202213 (European Stroke Network, www.europeanstrokenetwork.info/), The European Commission under the 7th Framework Programme – HEALTH – Collaborative Project Plasticise (Contract no 223524) – www.plasticise.eu, and The Spinal Cord Consortium of the Christopher and Dana Reeve Foundation.

References

Adesnik, H., Scanziani, M., 2010. Lateral competition for cortical space by layer-specific horizontal circuits. Nature 464, 1155–1160.

Adkins, D.L., Boychuk, J., Remple, M.S., Kleim, J.A., 2006. Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. J. Appl. Physiol. 101, 1776–1782.

Aguilar, J., Humanes-Valera, D., Alonso-Calvino, E., Yague, J.G., Moxon, K.A., Oliviero, A., Foffani, G., 2010. Spinal cord injury immediately changes the state of the brain. J. Neurosci. 30, 7528–7537.

Alexander, M.S., Anderson, K.D., Biering-Sorensen, F., Blight, A.R., Brannon, R., Bryce, T.N., Creasey, G., Catz, A., Curt, A., Donovan, W., Ditunno, J., Ellaway, P., Finnerup, N.B., Graves, D.E., Haynes, B.A., Heinemann, A.W., Jackson, A.B., Johnston, M.V., Kalpakjian, C.Z., Kleitman, N., Krassioukov, A., Krogh, K., Lammertse, D., Magasi, S., Mulcahey, M.J., Schurch, B., Sherwood, A., Steeves, J.D., Stiens, S., Tulsky, D.S., van Hedel, H.J., Whiteneck, G., 2009. Outcome measures in spinal cord injury: recent assessments and recommendations for future directions. Spinal Cord 47, 582–591.

Atwal, J.K., Pinkston-Gosse, J., Syken, J., Stawicki, S., Wu, Y., Shatz, C., Tessier-Lavigne, M., 2008. PirB is a functional receptor for myelin inhibitors of axonal regeneration. Science 322, 967–970.

Bareyre, F.M., Haudenschild, B., Schwab, M.E., 2002. Long-lasting sprouting and gene expression changes induced by the monoclonal antibody IN-1 in the adult spinal cord. J. Neurosci. 22, 7097–7110.

Bareyre, F.M., Kerschensteiner, M., Raineteau, O., Mettenleiter, T.C., Weinmann, O., Schwab, M.E., 2004. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nat. Neurosci. 7, 269–277.

Barriere, G., Leblond, H., Provencher, J., Rossignol, S., 2008. Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries. J. Neurosci. 28, 3976–3987.

Benson, M.D., Romero, M.I., Lush, M.E., Lu, Q.R., Henkemeyer, M., Parada, L.F., 2005. Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. Proc. Natl. Acad. Sci. USA 102, 10694–10699.

Bilston, L.E., Brown, J., 2007. Pediatric spinal injury type and severity are age and mechanism dependent. Spine Phila Pa 1976 32, 2339–2347.

Blochlinger, S., Weinmann, O., Schwab, M.E., Thallmair, M., 2001. Neuronal plasticity and formation of new synaptic contacts follow pyramidal lesions and neutralization of Nogo-A: a light and electron microscopic study in the pontine nuclei of adult rats. J. Comp. Neurol. 433, 426–436.

Brasil-Neto, J.P., Valls-Sole, J., Pascual-Leone, A., Cammarota, A., Amassian, V.E., Cracco, R., Maccabee, P., Cracco, J., Hallett, M., Cohen, L.G., 1993. Rapid modulation of human cortical motor outputs following ischaemic nerve block. Brain 116 (Pt 3), 511–525.

Bregman, B.S., Kunkel-Bagden, E., Schnell, L., Dai, H.N., Gao, D., Schwab, M.E., 1995. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. Nature 378, 498–501.

Bruehlmeier, M., Dietz, V., Leenders, K.L., Roelcke, U., Missimer, J., Curt, A., 1998. How does the human brain deal with a spinal cord injury? Eur. J. Neurosci. 10, 3918–3922.

Brus-Ramer, M., Carmel, J.B., Chakrabarty, S., Martin, J.H., 2007. Electrical stimulation of spared corticospinal axons augments connections with ipsilateral spinal motor circuits after injury. J. Neurosci. 27, 13793–13801.

Buffo, A., Zagrebelsky, M., Huber, A.B., Skerra, A., Schwab, M.E., Strata, P., Rossi, F., 2000. Application of neutralizing antibodies against NI-35/250 myelin-associated neurite growth inhibitory proteins to the adult rat cerebellum induces sprouting of uninjured purkinje cell axons. J. Neurosci. 20, 2275–2286.

Burns, S.P., Golding, D.G., Rolle Jr., W.A., Graziani, V., Ditunno Jr., J.F., 1997. Recovery of ambulation in motor-incomplete tetraplegia. Arch. Phys. Med. Rehabil. 78, 1169–1172.

Cafferty, W.B., Duffy, P., Huebner, E., Strittmatter, S.M., 2010. MAG and OMgp synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. J. Neurosci. 30, 6825–6837.

Cafferty, W.B., Strittmatter, S.M., 2006. The Nogo-Nogo receptor pathway limits a spectrum of adult CNS axonal growth. J. Neurosci. 26, 12242–12250.

Cai, L.L., Courtine, G., Fong, A.J., Burdick, J.W., Roy, R.R., Edgerton, V.R., 2006. Plasticity of functional connectivity in the adult spinal cord. Philos. Trans. R. Soc. Lond. B Biol. Sci. 361, 1635–1646.

Carmel, J.B., Berrol, L.J., Brus-Ramer, M., Martin, J.H., 2010. Chronic electrical stimulation of the intact corticospinal system after unilateral injury restores skilled locomotor control and promotes spinal axon outgrowth. J. Neurosci. 30, 10918–10926.

Caroni, P., Schwab, M.E., 1988. Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading. J. Cell Biol. 106, 1281–1288.

Cha, J., Heng, C., Reinkensmeyer, D.J., Roy, R.R., Edgerton, V.R., De Leon, R.D., 2007. Locomotor ability in spinal rats is dependent on the amount of activity imposed on the hindlimbs during treadmill training. J. Neurotrauma 24, 1000–1012.

Chen, M.S., Huber, A.B., van der Haar, M.E., Frank, M., Schnell, L., Spillmann, A.A., Christ, F., Schwab, M.E., 2000. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403, 434–439.

Choi, J.T., Vining, E.P., Mori, S., Bastian, A.J., 2010. Sensorimotor function and sensorimotor tracts after hemispherectomy. Neuropsychologia 48, 1192–1199.

Courtine, G., Gerasimenko, Y., van den Brand, R., Yew, A., Musienko, P., Zhong, H., Song, B., Ao, Y., Ichiyama, R.M., Lavrov, I., Roy, R.R., Sofroniew, M.V., Edgerton, V.R., 2009. Transformation of nonfunctional spinal circuits into functional states after the loss of brain input. Nat. Neurosci. 12, 1333–1342.

Courtine, G., Song, B., Roy, R.R., Zhong, H., Herrmann, J.E., Ao, Y., Qi, J., Edgerton, V.R., Sofroniew, M.V., 2008. Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. Nat. Med. 14, 69–74.

Curt, A., Alkadhi, H., Crelier, G.R., Boendermaker, S.H., Hepp-Reymond, M.C., Kollias, S.S., 2002a. Changes of non-affected upper limb cortical representation in paraplegic patients as assessed by fMRI. Brain 125, 2567–2578.

Curt, A., Bruehlmeier, M., Leenders, K.L., Roelcke, U., Dietz, V., 2002b. Differential effect of spinal cord injury and functional impairment on human brain activation. J. Neurotrauma 19, 43–51.

Dai, H., MacArthur, L., McAtee, M., Hockenbury, N., Tidwell, J.L., McHugh, B., Mansfield, K., Finn, T., Hamers, F.P., Bregman, B.S., 2009. Activity-based therapies to promote forelimb use after a cervical spinal cord injury. J. Neurotrauma 26, 1719–1732.

Darian-Smith, C., 2009. Synaptic plasticity, neurogenesis, and functional recovery after spinal cord injury. Neuroscientist 15, 149–165.

De Leon, R.D., Hodgson, J.A., Roy, R.R., Edgerton, V.R., 1998a. Full weight-bearing hindlimb standing following stand training in the adult spinal cat. J. Neurophysiol. 80, 83–91.

de Leon, R.D., Hodgson, J.A., Roy, R.R., Edgerton, V.R., 1998b. Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. J. Neurophysiol. 79, 1329–1340.

Delekate, A., Zagrebelsky, M., Kramer, S., Schwab, M.E., Korte, M., 2011. Nogo A restricts synaptic plasticity in the adult hippocampus on a fast time scale. Proc. Natl. Acad. Sci. USA 108, 2569–2574.

Dietz, V., 2010. Behavior of spinal neurons deprived of supraspinal input. Nat. Rev. Neurol. 6, 167–174.

Dietz, V., Colombo, G., 2004. Recovery from spinal cord injury—underlying mechanisms and efficacy of rehabilitation. Acta Neurochir. Suppl. 89, 95–100.

Dimou, L., Schnell, L., Montani, L., Duncan, C., Simonen, M., Schneider, R., Liebscher, T., Gullo, M., Schwab, M.E., 2006. Nogo-A-deficient mice reveal strain-dependent differences in axonal regeneration. J. Neurosci. 26, 5591–5603.

Dobkin, B.H., Havton, L.A., 2004. Basic advances and new avenues in therapy of spinal cord injury. Annu. Rev. Med. 55, 255–282.

Dobrossy, M.D., Dunnett, S.B., 2005. Optimising plasticity: environmental and training associated factors in transplant-mediated brain repair. Rev. Neurosci. 16, 1–21.

Please cite this article as: Starkey, M.L., Schwab, M.E., Anti-Nogo-A and training: Can one plus one equal three? Exp. Neurol. (2011), doi:10.1016/j.expneurol.2011.04.008

Domeniconi, M., Cao, Z., Spencer, T., Sivasankaran, R., Wang, K., Nikulina, E., Kimura, N., Cai, H., Deng, K., Gao, Y., He, Z., Filbin, M., 2002. Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. Neuron 35, 283–290.
Donoghue, J.P., Suner, S., Sanes, J.N., 1990. Dynamic organization of primary motor cortex output to target muscles in adult rats. II. Rapid reorganization following motor nerve lesions. Exp. Brain Res. 79, 492–503.
Edgerton, V.R., Courtine, G., Gerasimenko, Y.P., Lavrov, I., Ichiyama, R.M., Fong, A.J., Cai, L.L., Otoshi, C.K., Tillakaratne, N.J., Burdick, J.W., Roy, R.R., 2008. Training locomotor networks. Brain Res. Rev. 57, 241–254.
Edgerton, V.R., de Leon, R.D., Tillakaratne, N., Recktenwald, M.R., Hodgson, J.A., Roy, R.R., 1997. Use-dependent plasticity in spinal stepping and standing. Adv. Neurol. 72, 233–247.
Edgerton, V.R., Tillakaratne, N.J., Bigbee, A.J., de Leon, R.D., Roy, R.R., 2004. Plasticity of the spinal neural circuitry after injury. Annu. Rev. Neurosci. 27, 145–167.
Endo, T., Spenger, C., Tominaga, T., Brene, S., Olson, L., 2007. Cortical sensory map rearrangement after spinal cord injury: fMRI responses linked to Nogo signalling. Brain 130, 2951–2961.
Endo, T., Tominaga, T., Olson, L., 2009. Cortical changes following spinal cord injury with emphasis on the Nogo signaling system. Neuroscientist 15, 291–299.
Engesser-Cesar, C., Ichiyama, R.M., Nefas, A.L., Hill, M.A., Edgerton, V.R., Cotman, C.W., Anderson, A.J., 2007. Wheel running following spinal cord injury improves locomotor recovery and stimulates serotonergic fiber growth. Eur. J. Neurosci. 25, 1931–1939.
Fang, P.C., Barbay, S., Plautz, E.J., Hoover, E., Strittmatter, S.M., Nudo, R.J., 2010. Combination of NEP 1–40 treatment and motor training enhances behavioral recovery after a focal cortical infarct in rats. Stroke 41, 544–549.
Fouad, K., Metz, G.A., Merkler, D., Dietz, V., Schwab, M.E., 2000. Treadmill training in incomplete spinal cord injured rats. Behav. Brain Res. 115, 107–113.
Fouad, K., Pedersen, V., Schwab, M.E., Brosamle, C., 2001. Cervical sprouting of corticospinal fibers after thoracic spinal cord injury accompanies shifts in evoked motor responses. Curr. Biol. 11, 1766–1770.
Fournier, A.E., GrandPre, T., Gould, G., Wang, X., Strittmatter, S.M., 2002. Nogo and the Nogo-66 receptor. Prog. Brain Res. 137, 361–369.
Fournier, A.E., GrandPre, T., Strittmatter, S.M., 2001. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature 409, 341–346.
Freund, P., Schmidlin, E., Wannier, T., Bloch, J., Mir, A., Schwab, M.E., Rouiller, E.M., 2006. Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates. Nat. Med. 12, 790–792.
Freund, P., Schmidlin, E., Wannier, T., Bloch, J., Mir, A., Schwab, M.E., Rouiller, E.M., 2009. Anti-Nogo-A antibody treatment promotes recovery of manual dexterity after unilateral cervical lesion in adult primates—re-examination and extension of behavioral data. Eur. J. Neurosci. 29, 983–996.
Freund, P., Wannier, T., Schmidlin, E., Bloch, J., Mir, A., Schwab, M.E., Rouiller, E.M., 2007. Anti-Nogo-A antibody treatment enhances sprouting of corticospinal axons rostral to a unilateral cervical spinal cord lesion in adult macaque monkey. J. Comp. Neurol. 502, 644–659.
Frigon, A., Rossignol, S., 2006. Functional plasticity following spinal cord lesions. Prog. Brain Res. 157, 231–260.
Garcia-Alias, G., Barkhuysen, S., Buckle, M., Fawcett, J.W., 2009. Chondroitinase ABC treatment opens a window of opportunity for task-specific rehabilitation. Nat. Neurosci. 12, 1145–1151.
Ghosh, A., Haiss, F., Sydekum, E., Schneider, R., Gullo, M., Wyss, M.T., Mueggler, T., Baltes, C., Rudin, M., Weber, B., Schwab, M.E., 2010. Rewiring of hindlimb corticospinal neurons after spinal cord injury. Nat. Neurosci. 13, 97–104.
Ghosh, A., Sydekum, E., Haiss, F., Peduzzi, S., Zorner, B., Schneider, R., Baltes, C., Rudin, M., Weber, B., Schwab, M.E., 2009. Functional and anatomical reorganization of the sensory-motor cortex after incomplete spinal cord injury in adult rats. J. Neurosci. 29, 12210–12219.
Girgis, J., Merrett, D., Kirkland, S., Metz, G.A., Verge, V., Fouad, K., 2007. Reaching training in rats with spinal cord injury promotes plasticity and task specific recovery. Brain 130, 2993–3003.
Goldberg, J.L., Vargas, M.E., Wang, J.T., Mandemakers, W., Oster, S.F., Sretavan, D.W., Barres, B.A., 2004. An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. J. Neurosci. 24, 4989–4999.
Gonzenbach, R.R., Gasser, P., Zorner, B., Hochreutener, E., Dietz, V., Schwab, M.E., 2010. Nogo-A antibodies and training reduce muscle spasms in spinal cord-injured rats. Ann. Neurol. 68, 48–57.
GrandPre, T., Li, S., Strittmatter, S.M., 2002. Nogo-66 receptor antagonist peptide promotes axonal regeneration. Nature 417, 547–551.
GrandPre, T., Nakamura, F., Vartanian, T., Strittmatter, S.M., 2000. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 403, 439–444.
Grasso, R., Ivanenko, Y.P., Zago, M., Molinari, M., Scivoletto, G., Lacquaniti, F., 2004. Recovery of forward stepping in spinal cord injured patients does not transfer to untrained backward stepping. Exp. Brain Res. 157, 377–382.
Harel, N., Song, K.H., Tang, X., Strittmatter, S., 2010. Nogo receptor deletion and multimodal exercise improve distinct aspects of recovery in cervical spinal cord injury. J. Neurotrauma 27, 2055–2066.
Harmsen, P., Lappas, G., Rosengren, A., Wilhelmsen, L., 2006. Long-term risk factors for stroke: twenty-eight years of follow-up of 7457 middle-aged men in Goteborg, Sweden. Stroke 37, 1663–1667.
Hendry, S.H., Jones, E.G., 1986. Reduction in number of immunostained GABAergic neurones in deprived-eye dominance columns of monkey area 17. Nature 320, 750–753.
Hess, G., Donoghue, J.P., 1994. Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. J. Neurophysiol. 71, 2543–2547.
Hess, G., Donoghue, J.P., 1996. Long-term potentiation and long-term depression of horizontal connections in rat motor cortex. Acta Neurobiol. Exp. Wars 56, 397–405.
Holme, I., Tonstad, S., Sogaard, A.J., Larsen, P.G., Haheim, L.L., 2007. Leisure time physical activity in middle age predicts the metabolic syndrome in old age: results of a 28-year follow-up of men in the Oslo study. BMC Public Health 7, 154.
Hsu, J.E., Jones, T.A., 2006. Contralesional neural plasticity and functional changes in the less-affected forelimb after large and small cortical infarcts in rats. Exp. Neurol. 201, 479–494.
Ichiyama, R.M., Broman, J., Roy, R.R., Zhong, H., Edgerton, V.R., Havton, L.A., 2011. Locomotor training maintains normal inhibitory influence on both alpha- and gamma-motoneurons after neonatal spinal cord transection. J. Neurosci. 31, 26–33.
Jacobs, K.M., Donoghue, J.P., 1991. Reshaping the cortical motor map by unmasking latent intracortical connections. Science 251, 944–947.
Jakob, W., Wirz, M., van Hedel, H.J., Dietz, V., 2009. Difficulty of elderly SCI subjects to translate motor recovery–"body function"–into daily living activities. J. Neurotrauma 26, 2037–2044.
Ji, B., Li, M., Wu, W.T., Yick, L.W., Lee, X., Shao, Z., Wang, J., So, K.F., McCoy, J.M., Pepinsky, R.B., Mi, S., Relton, J.K., 2006. (LINGO-1 antagonist promotes functional recovery and axonal sprouting after spinal cord injury. Mol. Cell Neurosci. 33, 311–320.
Joset, A., Dodd, D.A., Halegoua, S., Schwab, M.E., 2010. Pincher-generated Nogo-A endosomes mediate growth cone collapse and retrograde signaling. J. Cell Biol. 188, 271–285.
Kaas, J.H., 1991. Plasticity of sensory and motor maps in adult mammals. Annu. Rev. Neurosci. 14, 137–167.
Kanagal, S.G., Muir, G.D., 2009. Task-dependent compensation after pyramidal tract and dorsolateral spinal lesions in rats. Exp. Neurol. 216, 193–206.
Kapfhammer, J.P., Schwab, M.E., 1994. Increased expression of the growth-associated protein GAP-43 in the myelin-free rat spinal cord. Eur. J. Neurosci. 6, 403–411.
Kerschensteiner, M., Bareyre, F.M., Buddeberg, B.S., Merkler, D., Stadelmann, C., Bruck, W., Misgeld, T., Schwab, M.E., 2004. Remodeling of axonal connections contributes to recovery in an animal model of multiple sclerosis. J. Exp. Med. 200, 1027–1038.
Kim, B.G., Dai, H.N., McAtee, M., Bregman, B.S., 2008. Modulation of dendritic spine remodeling in the motor cortex following spinal cord injury: effects of environmental enrichment and combinatorial treatment with transplants and neurotrophin-3. J. Comp. Neurol. 508, 473–486.
Kim, J.E., Li, S., GrandPre, T., Qiu, D., Strittmatter, S.M., 2003. Axon regeneration in young adult mice lacking Nogo-A/B. Neuron 38, 187–199.
Kim, J.E., Liu, B.P., Park, J.H., Strittmatter, S.M., 2004. Nogo-66 receptor prevents raphespinal and rubrospinal axon regeneration and limits functional recovery from spinal cord injury. Neuron 44, 439–451.
Kleim, J.A., Hogg, T.M., VandenBerg, P.M., Cooper, N.R., Bruneau, R., Remple, M., 2004. Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. J. Neurosci. 24, 628–633.
Krajacic, A., Weishaupt, N., Girgis, J., Tetzlaff, W., Fouad, K., 2010. Training-induced plasticity in rats with cervical spinal cord injury: effects and side effects. Behav. Brain Res. 214, 323–331.
Kuerzi, J., Brown, E.H., Shum-Siu, A., Siu, A., Burke, D., Morehouse, J., Smith, R.R., Magnuson, D.S., 2010. Task-specificity vs. ceiling effect: step-training in shallow water after spinal cord injury. Exp. Neurol. 224, 178–187.
Lankhorst, A.J., ter Laak, M.P., van Laar, T.J., van Meeteren, N.L., de Groot, J.C., Schrama, L.H., Hamers, F.P., Gispen, W.H., 2001. Effects of enriched housing on functional recovery after spinal cord contusive injury in the adult rat. J. Neurotrauma 18, 203–215.
Lee, J.K., Geoffroy, C.G., Chan, A.F., Tolentino, K.E., Crawford, M.J., Leal, M.A., Kang, B., Zheng, B., 2010. Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. Neuron 66, 663–670.
Lee, J.K., Kim, J.E., Sivula, M., Strittmatter, S.M., 2004. Nogo receptor antagonism promotes stroke recovery by enhancing axonal plasticity. J. Neurosci. 24, 6209–6217.
Li, S., Liu, B.P., Budel, S., Li, M., Ji, B., Walus, L., Li, W., Jirik, A., Rabacchi, S., Choi, E., Worley, D., Sah, D.W., Pepinsky, B., Lee, D., Relton, J., Strittmatter, S.M., 2004. Blockade of Nogo-66, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein by soluble Nogo-66 receptor promotes axonal sprouting and recovery after spinal injury. J. Neurosci. 24, 10511–10520.
Li, S., Strittmatter, S.M., 2003. Delayed systemic Nogo-66 receptor antagonist promotes recovery from spinal cord injury. J. Neurosci. 23, 4219–4227.
Liebscher, T., Schnell, L., Schnell, D., Scholl, J., Schneider, R., Gullo, M., Fouad, K., Mir, A., Rausch, M., Kindler, D., Hamers, F.P., Schwab, M.E., 2005. Nogo-A antibody improves regeneration and locomotion of spinal cord-injured rats. Ann. Neurol. 58, 706–719.
Little, J.W., Ditunno Jr., J.F., Stiens, S.A., Harris, R.M., 1999. Incomplete spinal cord injury: neuronal mechanisms of motor recovery and hyperreflexia. Arch. Phys. Med. Rehabil. 80, 587–599.
Liu, B.P., Fournier, A., GrandPre, T., Strittmatter, S.M., 2002. Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. Science 297, 1190–1193.
Lovely, R.G., Gregor, R.J., Roy, R.R., Edgerton, V.R., 1986. Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. Exp. Neurol. 92, 421–435.
Low, K., Culbertson, M., Bradke, F., Tessier-Lavigne, M., Tuszynski, M.H., 2008. Netrin-1 is a novel myelin-associated inhibitor to axon growth. J. Neurosci. 28, 1099–1108.
Maier, I.C., Baumann, K., Thallmair, M., Weinmann, O., Scholl, J., Schwab, M.E., 2008. Constraint-induced movement therapy in the adult rat after unilateral corticospinal tract injury. J. Neurosci. 28, 9386–9403.
Maier, I.C., Ichiyama, R.M., Courtine, G., Schnell, L., Lavrov, I., Edgerton, V.R., Schwab, M.E., 2009. Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. Brain 132, 1426–1440.

Please cite this article as: Starkey, M.L., Schwab, M.E., Anti-Nogo-A and training: Can one plus one equal three? Exp. Neurol. (2011), doi:10.1016/j.expneurol.2011.04.008

Marsh, B.C., Astill, S.L., Utley, A., Ichiyama, R.M., 2010. Movement rehabilitation after spinal cord injuries: emerging concepts and future directions. Brain Res. Bull. 84, 327–336.
Martin, J.H., Choy, M., Pullman, S., Meng, Z., 2004. Corticospinal system development depends on motor experience. J. Neurosci. 24, 2122–2132.
Martin, J.H., Engber, D., Meng, Z., 2005. Effect of forelimb use on postnatal development of the forelimb motor representation in primary motor cortex of the cat. J. Neurophysiol. 93, 2822–2831.
Martin, J.H., Kably, B., Hacking, A., 1999. Activity-dependent development of cortical axon terminations in the spinal cord and brain stem. Exp. Brain Res. 125, 184–199.
Martinez, M., Brezun, J.M., Zennou-Azogui, Y., Baril, N., Xerri, C., 2009. Sensorimotor training promotes functional recovery and somatosensory cortical map reactivation following cervical spinal cord injury. Eur. J. Neurosci. 30, 2356–2367.
McKerracher, L., David, S., Jackson, D.L., Kottis, V., Dunn, R.J., Braun, P.E., 1994. Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron 13, 805–811.
Merkler, D., Metz, G.A., Raineteau, O., Dietz, V., Schwab, M.E., Fouad, K., 2001. Locomotor recovery in spinal cord-injured rats treated with an antibody neutralizing the myelin-associated neurite growth inhibitor Nogo-A. J. Neurosci. 21, 3665–3673.
Merzenich, M.M., Kaas, J.H., Wall, J., Nelson, R.J., Sur, M., Felleman, D., 1983a. Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. Neuroscience 8, 33–55.
Merzenich, M.M., Kaas, J.H., Wall, J.T., Sur, M., Nelson, R.J., Felleman, D.J., 1983b. Progression of change following median nerve section in the cortical representation of the hand in areas 3b and 1 in adult owl and squirrel monkeys. Neuroscience 10, 639–665.
Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B., Crowell, T., Cate, R.L., McCoy, J.M., Pepinsky, R.B., 2004. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat. Neurosci. 7, 221–228.
Montani, L., Gerrits, B., Gehrig, P., Kempf, A., Dimou, L., Wollscheid, B., Schwab, M.E., 2009. Neuronal Nogo-A modulates growth cone motility via Rho-GTP/LIMK1/cofilin in the unlesioned adult nervous system. J. Biol. Chem. 284, 10793–10807.
Moreau-Fauvarque, C., Kumanogoh, A., Camand, E., Jaillard, C., Barbin, G., Boquet, I., Love, C., Jones, E.Y., Kikutani, H., Lubetzki, C., Dusart, I., Chedotal, A., 2003. The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J. Neurosci. 23, 9229–9239.
Mullner, A., Gonzenbach, R.R., Weinmann, O., Schnell, L., Liebscher, T., Schwab, M.E., 2008. Lamina-specific restoration of serotonergic projections after Nogo-A antibody treatment of spinal cord injury in rats. Eur. J. Neurosci. 27, 326–333.
Multon, S., Franzen, R., Poirrier, A.L., Scholtes, F., Schoenen, J., 2003. The effect of treadmill training on motor recovery after a partial spinal cord compression-injury in the adult rat. J. Neurotrauma 20, 699–706.
Nash, M., Pribiag, H., Fournier, A.E., Jacobson, C., 2009. Central nervous system regeneration inhibitors and their intracellular substrates. Mol. Neurobiol. 40, 224–235.
Nishimura, Y., Isa, T., 2009. Compensatory changes at the cerebral cortical level after spinal cord injury. Neuroscientist 15, 436–444.
Oertle, T., Schwab, M.E., 2003. Nogo and its paRTNers. Trends Cell Biol. 13, 187–194.
Oertle, T., van der Haar, M.E., Bandtlow, C.E., Robeva, A., Burfeind, P., Buss, A., Huber, A.B., Simonen, M., Schnell, L., Brosamle, C., Kaupmann, K., Vallon, R., Schwab, M.E., 2003. Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. J. Neurosci. 23, 5393–5406.
Papadopoulos, C.M., Tsai, S.Y., Alsbiei, T., O'Brien, T.E., Schwab, M.E., Kartje, G.L., 2002. Functional recovery and neuroanatomical plasticity following middle cerebral artery occlusion and IN-1 antibody treatment in the adult rat. Ann. Neurol. 51, 433–441.
Park, J.B., Yiu, G., Kaneko, S., Wang, J., Chang, J., He, X.L., Garcia, K.C., He, Z., 2005. A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. Neuron 45, 345–351.
Piecharka, D.M., Kleim, J.A., Whishaw, I.Q., 2005. Limits on recovery in the corticospinal tract of the rat: partial lesions impair skilled reaching and the topographic representation of the forelimb in motor cortex. Brain Res. Bull. 66, 203–211.
Raiker, S.J., Lee, H., Baldwin, K.T., Duan, Y., Shrager, P., Giger, R.J., 2010. Oligodendrocyte-myelin glycoprotein and Nogo negatively regulate activity-dependent synaptic plasticity. J. Neurosci. 30, 12432–12445.
Raineteau, O., Fouad, K., Bareyre, F.M., Schwab, M.E., 2002. Reorganization of descending motor tracts in the rat spinal cord. Eur. J. Neurosci. 16, 1761–1771.
Ramer, M.S., 2010. Endogenous neurotrophins and plasticity following spinal deafferentation. Exp. Neurol. [Epub ahead of print].
Rioult-Pedotti, M.S., Donoghue, J.P., Dunaevsky, A., 2007. Plasticity of the synaptic modification range. J. Neurophysiol. 98, 3688–3695.
Rosenzweig, E.S., Courtine, G., Jindrich, D.L., Brock, J.H., Ferguson, A.R., Strand, S.C., Nout, Y.S., Roy, R.R., Miller, D.M., Beattie, M.S., Havton, L.A., Bresnahan, J.C., Edgerton, V.R., Tuszynski, M.H., 2010. Extensive spontaneous plasticity of corticospinal projections after primate spinal cord injury. Nat. Neurosci. 13, 1505–1510.
Rossignol, S., Bouyer, L., Barthelemy, D., Langlet, C., Leblond, H., 2002. Recovery of locomotion in the cat following spinal cord lesions. Brain Res. Brain Res. Rev. 40, 257–266.
Salimi, I., Martin, J.H., 2004. Rescuing transient corticospinal terminations and promoting growth with corticospinal stimulation in kittens. J. Neurosci. 24, 4952–4961.
Sanes, J.N., Suner, S., Lando, J.F., Donoghue, J.P., 1988. Rapid reorganization of adult rat motor cortex somatic representation patterns after motor nerve injury. Proc. Natl. Acad. Sci. USA 85, 2003–2007.
Savio, T., Schwab, M.E., 1989. Rat CNS white matter, but not gray matter, is nonpermissive for neuronal cell adhesion and fiber outgrowth. J. Neurosci. 9, 1126–1133.
Savio, T., Schwab, M.E., 1990. Lesioned corticospinal tract axons regenerate in myelin-free rat spinal cord. Proc. Natl. Acad. Sci. USA 87, 4130–4133.
Schmidlin, E., Wannier, T., Bloch, J., Rouiller, E.M., 2004. Progressive plastic changes in the hand representation of the primary motor cortex parallel incomplete recovery from a unilateral section of the corticospinal tract at cervical level in monkeys. Brain Res. 1017, 172–183.
Schnell, L., Schwab, M.E., 1990. Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. Nature 343, 269–272.
Schucht, P., Raineteau, O., Schwab, M.E., Fouad, K., 2002. Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the rat spinal cord. Exp. Neurol. 176, 143–153.
Schwab, M.E., 2004. Nogo and axon regeneration. Curr. Opin. Neurobiol. 14, 118–124.
Schwab, M.E., 2010. Functions of Nogo proteins and their receptors in the nervous system. Nat. Rev. Neurosci. 11, 799–811.
Simonen, M., Pedersen, V., Weinmann, O., Schnell, L., Buss, A., Ledermann, B., Christ, F., Sansig, G., van der Putten, H., Schwab, M.E., 2003. Systemic deletion of the myelin-associated outgrowth inhibitor Nogo-A improves regenerative and plastic responses after spinal cord injury. Neuron 38, 201–211.
Singh, A., Murray, M., Houle, J., 2010. A training paradigm to enhance motor recovery in contused rats: effects of staircase training. Neurorehabil. Neural Repair.
Smith, R.R., Shum-Siu, A., Baltzley, R., Bunger, M., Baldini, A., Burke, D.A., Magnuson, D.S., 2006. Effects of swimming on functional recovery after incomplete spinal cord injury in rats. J. Neurotrauma 23, 908–919.
Spiess, M.R., Muller, R.M., Rupp, R., Schuld, C., van Hedel, H.J., 2009. Conversion in ASIA impairment scale during the first year after traumatic spinal cord injury. J. Neurotrauma 26, 2027–2036.
Takahashi, M., Vattanajun, A., Umeda, T., Isa, K., Isa, T., 2009. Large-scale reorganization of corticofugal fibers after neonatal hemidecortication for functional restoration of forelimb movements. Eur. J. Neurosci. 30, 1878–1887.
Teasell, R., Bitensky, J., Foley, N., Bayona, N.A., 2005. Training and stimulation in post stroke recovery brain reorganization. Top. Stroke Rehabil. 12, 37–45.
Thallmair, M., Metz, G.A., Z'Graggen, W.J., Raineteau, O., Kartje, G.L., Schwab, M.E., 1998. Neurite growth inhibitors restrict plasticity and functional recovery following corticospinal tract lesions. Nat. Neurosci. 1, 124–131.
Tillakaratne, N.J., Guu, J.J., de Leon, R.D., Bigbee, A.J., London, N.J., Zhong, H., Ziegler, M.D., Joynes, R.L., Roy, R.R., Edgerton, V.R., 2010. Functional recovery of stepping in rats after a complete neonatal spinal cord transection is not due to regrowth across the lesion site. Neuroscience 166, 23–33.
van Hedel, H.J., Dietz, V., 2004. The influence of age on learning a locomotor task. Clin. Neurophysiol. 115, 2134–2143.
Venkatesh, K., Chivatakarn, O., Sheu, S.S., Giger, R.J., 2007. Molecular dissection of the myelin-associated glycoprotein receptor complex reveals cell type-specific mechanisms for neurite outgrowth inhibition. J. Cell Biol. 177, 393–399.
Wang, K.C., Kim, J.A., Sivasankaran, R., Segal, R., He, Z., 2002a. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. Nature 420, 74–78.
Wang, K.C., Koprivica, V., Kim, J.A., Sivasankaran, R., Guo, Y., Neve, R.L., He, Z., 2002b. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature 417, 941–944.
Wang, M.Y., Hoh, D.J., Leary, S.P., Griffith, P., McComb, J.G., 2004. High rates of neurological improvement following severe traumatic pediatric spinal cord injury. Spine Phila Pa 1976 29, 1493–1497 discussion E1266.
Willi, R., Aloy, E.M., Yee, B.K., Feldon, J., Schwab, M.E., 2009. Behavioral characterization of mice lacking the neurite outgrowth inhibitor Nogo-A. Genes Brain Behav. 8, 181–192.
Wurbel, H., 2001. Ideal homes? Housing effects on rodent brain and behaviour. Trends Neurosci. 24, 207–211.
Xu, T., Yu, X., Perlik, A.J., Tobin, W.F., Zweig, J.A., Tennant, K., Jones, T., Zuo, Y., 2009. Rapid formation and selective stabilization of synapses for enduring motor memories. Nature 462, 915–919.
Yiu, G., He, Z., 2006. Glial inhibition of CNS axon regeneration. Nat. Rev. Neurosci. 7, 617–627.
Z'Graggen, W.J., Metz, G.A., Kartje, G.L., Thallmair, M., Schwab, M.E., 1998. Functional recovery and enhanced corticofugal plasticity after unilateral pyramidal tract lesion and blockade of myelin-associated neurite growth inhibitors in adult rats. J. Neurosci. 18, 4744–4757.
Zagrebelsky, M., Buffo, A., Skerra, A., Schwab, M.E., Strata, P., Rossi, F., 1998. Retrograde regulation of growth-associated gene expression in adult rat Purkinje cells by myelin-associated neurite growth inhibitory proteins. J. Neurosci. 18, 7912–7929.
Zagrebelsky, M., Schweigreiter, R., Bandtlow, C.E., Schwab, M.E., Korte, M., 2010. Nogo-A stabilizes the architecture of hippocampal neurons. J. Neurosci. 30, 13220–13234.
Zheng, B., Ho, C., Li, S., Keirstead, H., Steward, O., Tessier-Lavigne, M., 2003. Lack of enhanced spinal regeneration in Nogo-deficient mice. Neuron 38, 213–224.
Zorner, B., Filli, L., Starkey, M.L., Gonzenbach, R., Kasper, H., Rothlisberger, M., Bolliger, M., Schwab, M.E., 2010. Profiling locomotor recovery: comprehensive quantification of impairments after CNS damage in rodents. Nat. Methods 7, 701–708.

Please cite this article as: Starkey, M.L., Schwab, M.E., Anti-Nogo-A and training: Can one plus one equal three? Exp. Neurol. (2011), doi:10.1016/j.expneurol.2011.04.008