Plasticity in Sublesionally Located Neurons Following Spinal Cord Injury

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Submitted 5 June 2007; accepted in final form 17 September 2007

INTRODUCTION

Spinal cord injury (SCI) induces devastating damages leading to a permanent loss of sensory and voluntary motor functions. Several mechanisms underlying the poor spontaneous repair and regenerative capabilities of the spinal cord after trauma have been identified (e.g., McKerracher and David 2004). In turn, growing evidence shows that neurons located caudal to the lesion (sublesional neurons) generally remain alive and, to some extent, functional. Sublesional neurons have been shown to undergo a number of plastic and adaptive changes within a few days to a few months post-trauma. However, it remains unclear how these changes are associated with functional adaptation and how training can modulate sublesional plasticity post-SCI.

Involuntary and spontaneously occurring locomotor-like movements are among the consequences of functional adaptations found under some circumstances in chronic SCI patients (Calancie et al. 1994). A partial recovery of ambulation per se has also been found using adapted training methods in incomplete SCI subjects (Dobkin et al. 2006; Wormig and Muller 1992). Outstanding recovery levels have even been reported using intensive treadmill training with weight-support assistance and afferent stimulation in complete spinal cord transected cats (Barbeau and Rossignol 1987; Barbeau et al. 1993; Belanger et al. 1996; Chau et al. 1998; Lovely et al. 1986).

Functional changes in incomplete or complete SCI mammals have been attributed generally, not to sprouting and neuronal repair, but to spontaneous plasticity and/or training-induced cell property changes caudal to the spinal cord injury. This hypothesis is supported by the existence of a network of “locomotor” neurons in the lumbar spinal cord called the central pattern generator or CPG (i.e., note that various related theoretical models have been proposed—e.g., half-centers or unit burst generators), which can be made to produce, in some conditions, the basic signals for walking in the absence of inputs from the brain and the peripheral sensory system (Grillner and Zangger 1979; also see next section with in vitro data). For early evidence in spinal cats after partial deafferentation or “narcosis progression,” also see Brown (1911, 1914).

However, the detailed cellular changes within the lumbar spinal cord or the CPG that may underlie the type of recovery found sometimes in incomplete SCI patients or even in complete SCI animals remain poorly understood (for spontaneous recovery and plasticity associated with descending tract sprouting, see e.g. Ballermann and Fouad 2006; Bareyre et al. 2004). It is hoped that the next sections, which include an updated list of the sublesional cell property changes reported after a complete spinal cord transection and the current evidence suggesting their contribution to spontaneous or therapy-induced locomotor function recovery, will provide insights into these mechanisms.

CPG-associated changes: functional evidence

The existence of a CPG and its role in locomotor pattern and rhythm generation have been shown in all classes of vertebrate species. In the 1970s, Grillner was the first to demonstrate its existence in mammalian species, when reporting that locomotor activity in hindlimb motor nerves could be acutely induced after L-DOPA administration (intravenous) in deafferented and low-thoracic spinal cord transected cats (Grillner and Zangger 1979). These experiments have shown that a network of sublesional neurons (caudal to the 12th thoracic segment) had the capacity of generating the basic signals for walking even in the complete absence of supraspinal inputs and phasic inputs from peripheral nerves. A clear demonstration of its existence in humans has remained difficult because only data from an entirely deafferented and complete SCI patient would satisfy all criteria. Nonetheless, in the 1990s, Calancie reported evidence suggesting the existence of a CPG in a chronic tetraplegic patient (Calancie et al. 1994). Although not completely SCI, that patient was shown, once lying down on a table, to spontaneously display locomotor-like movements in the lower limbs. More convincing data were reported a few years later by...
Dimitrijevic, who showed locomotor-like movements induced by epidural stimulation of the spinal cord (i.e., lumbar segments) in complete paraplegic SCI subjects (Dimitrijevic et al. 1998). Those results strongly suggested the existence of critical CPG elements in the lumbar or thoraco-lumbar area of the spinal cord in humans. Although a contribution from peripheral inputs (e.g., proprioceptors, skin receptors, etc.) was possible, this series of experiments has clearly shown that spinal stepping is possible even in the complete absence of inputs from the brain. During the same period, automatic air-stepping (in completely suspended subjects) was successfully induced in healthy persons after tonic muscle vibration, which may also partially support the idea of a CPG (activated by afferent inputs) in humans (Gurfinkel et al. 1998). Before those studies, preliminary evidence of a CPG was found in a complete SCI patient who developed myoclonus and leg movements resembling locomotion (e.g., low frequency, rhythmic, and bilaterally alternating; Bussel et al. 1988; for additional evidence also see Illis 1995; Nicol et al. 1995).

On the other hand, numerous studies have shown that regular training on a treadmill can improve ambulation in incomplete SCI individuals, suggesting a role for training-induced plasticity and reorganization of sublesional neuronal networks in functional recovery (Behrman and Harkema 2000; Fung et al. 1990; Stewart et al. 1991; Wainberg et al. 1990). This is also supported by the work of Dimitrijevic and colleagues (1998) who showed that epidural stimulation-induced CPG activation is facilitated in SCI subjects that have received regular locomotor training. Other studies support the idea of training-induced plasticity changes sublesionally. Indeed, using weight-support-assistance or walking devices, incomplete SCI patients were found to significantly improve their level of ambulation (Norman et al. 1995; Wernig and Muller 1992; see also Dobkin et al. 2006). Combining both simultaneously epidural stimulation and body-weight supported treadmill training (BWSTT) has also been shown to lead to significant locomotor function recovery (Herman et al. 2002). Although interesting for incomplete SCI patients, these methods (e.g., regular training, BWSTT) have generally been less successful at inducing locomotor movements in complete SCI individuals. In turn, this may suggest that spontaneous- or training-induced changes require either 1) some spared descending fibers from the brain or 2) to be combined with other approaches (e.g., epidural stimulation or other CPG-activating means), to sufficiently increase CPG excitation and thus to trigger its activity and locomotor movements in complete SCI patients.

Recent studies with animal models of paraplegia have begun to provide insights that improve our understanding of sublesional plasticity and cell property changes post-SCI. For instance, using adapted methods for assessing even weak hindlimb movements, some spontaneous locomotor recovery was found to occur within a few weeks post-surgery in complete spinal cord transected mice (Guertin 2005b). Different levels of spontaneous recovery have also been reported in various strains (higher levels in CD1 than in C57BL/6 or BALB/C mice), showing that genetic backgrounds may influence spontaneously occurring plasticity in sublesional networks (i.e., increased CPG excitability; Lapointe et al. 2006).

The hypothesis of an increase of CPG excitability (i.e., due to increased excitation or decreased inhibition within the network or appropriate activation of CPG elements) in chronic SCI animals is also supported by recent in vitro data. Norreel and colleagues have shown that relatively low concentrations of bath-applied N-methyl-D-aspartate (NMDA) is sufficient to induce fictive locomotion in isolated spinal cords from 1-wk-paraplegic neonatal rats but not from control, nonparaplegic animals (Norreel et al. 2003). These results have provided additional evidence suggesting that CPG excitability is spontaneously increased soon after trauma (e.g., at $\geq 1$ wk). The authors have discussed the possibility that changes in excitability may arise from a decrease in GABAergic inhibition or an increase in excitation (e.g., increased excitatory receptor expression; Norreel et al. 2003). The latter possibility is supported by in vivo results from our laboratory showing that spinal stepping movements induced pharmacologically using CPG-activating excitatory drugs (e.g., 5-HT$_{1A}$/receptor agonists) are enhanced in complete paraplegic mice tested at 7 days compared with 3 days post-transsection (unpublished data).

**CPG-associated changes: cellular evidence**

Growing evidence indicates that an association may be made between some of the sublesional cellular adaptive changes post-trauma and a spontaneous or drug- and/or training-induced recovery of locomotor functions in chronic SCI animals. For instance, recent experiments have shown that the expression of some immediate early genes (IEGs) is up- or down-regulated in sublesional spinal cord areas within a few hours to a few days post-trauma. Landry and colleagues reported in low-thoracic spinal cord transected mice that $c$-$f$-$o$-s and $n$-or-1 were respectively increased and decreased in L1–L2 dorsal horn and intermediate zone areas (Landry et al. 2006b). Increased fos-immunoreactive levels were also found as soon as at $2.5 \ h$ post-trauma in sublesional segments (low-thoracic and lumbar laminae I–IV, VII, VIII, and X) of high-thoracic or cervical spinal cord transected rats (Ruggiero et al. 1997). Because these same lumbar cord segments (specifically L1–L2 in mice) have been shown to contain critical CPG elements (Nishimaru and Kudo 2000) and, given that IEGs are well known for their role in CNS development and plasticity (Ginty et al. 1992), spontaneous changes in IEG expression (i.e., specifically $c$-$f$-$o$-s and $n$-or-1) in L1–L2 segments may reasonably be considered as among the first cellular events associated with cell property changes and increased CPG excitability post-SCI (see discussion in Landry et al. 2006b).

Further supporting the idea of a role in plasticity and functional recovery, changes in IEG expression post-trauma timely correspond with a spontaneous return of “reflex” hindlimb movements in complete paraplegic mice. Small-amplitude nonlocomotor movements and locomotor-like movements were found generally to occur spontaneously after 7 and 14 days, respectively, following a complete transection of the spinal cord between the 9th and the 10th thoracic vertebrae in adult CD1 mice (Guertin 2005a,b; Lapointe et al. 2006). Because significant changes in $c$-$f$-$o$-s and $n$-or-1 expression were also found at 3, 7, and 14 days post-transsection; this provides evidence suggesting their role in the spontaneous hindlimb movement recovery found in complete paraplegic mice. However, it is also important to mention that some of these changes in the spinal cord may also be associated with phenomena other than plasticity or reorganization. For instance, $c$-$f$-$o$-s has been used as an activity-depen-
dent marker shown to be expressed in the spinal cord during locomotor activity (Dai et al. 2005; Jasmin et al. 1994) and in models investigating pain (e.g., Bai et al. 2007; Cruz et al. 2007; Pinto et al. 2007). Regarding nor-1 expression, it has also been associated with transient global ischemia in rats (Kim et al. 2006).

Numerous regulatory changes are likely to be subsequently activated after IEG alterations. These may include tumor necrosis factor-alpha (TNF-α) and preprodynorphin because their expression was found to display increased levels in lumbar segments after several hours to several days post-transection (Yakovlev and Faden 1994). Increased levels of nitric oxide synthase (NOS) expression were also found in lumbar segments at 1 and 7 days in low-thoracic hemisected rats (Lukacova et al. 2006). Byrnes and colleagues (2006) also reported changes in gene expression [C1q, Galectin-3, and p22(phox)] distally from the epicenter that peak at 3 and 7 days in mild, moderate, or severe SCI rats. Substantial changes in gene expression pattern have been reported several days to several weeks after injury in the whole spinal cord (Ma et al. 2006) or in areas above the lesion (Schmitt et al. 2006). Although a precise role for most of these regulatory and gene expression changes in functional recovery remains unknown, scientists generally agree that an association may be made between their alterations and plasticity, reorganization, and cell property changes in distally located networks (Chi et al. 1993; Ruggiero et al. 1997; Yakovlev and Faden 1994). A role for some of these changes (e.g., with TNF-α) in neuropathic pain (e.g., Peng et al. 2006) or inflammation (e.g., Pineau and Lacroix 2007) may also exist.

Other elements may be considered as good candidates for plasticity and increased CPG excitability post-trauma. For instance, transmembranal receptors such as glycine receptors have been found to display increased expression levels in lumbar spinal cord segments 3 mo after a low-thoracic transection in rats (Edgerton et al. 2001). Interestingly, this spontaneous change was found to be modulated by training because a return to normal levels was observed after 3 mo of regular treadmill training (with weight-support-assistance; Edgerton et al. 2001). Similar spontaneous changes and training-induced modulation were reported with GAD-67 (enzyme in γ-aminobutyric acid synthesis) in complete low-thoracic transected cats (Tillakaratne et al. 2002). This increase of GAD-67 expression was specifically detected in lumbar dorsal horn and intermediate zone areas.

Serotonin (5-HT) and noradrenaline receptor systems have also been shown to undergo important changes after SCI. Increased 5-HT1A receptor expression was revealed using autoradiography with [3H]8-OH-DPAT in lumbar spinal cord segments of chronic paraplegic cats (Giroux et al. 1999). Again, changes in several laminar areas including the intermediate zone (surrounding the central canal) were reported. Increased autoradiographic levels associated with α1 and α2 adrenoceptors were also observed in similar segmental and laminar areas of the spinal cord in low-thoracic transected cats (Giroux et al. 1999). Interestingly, using in situ hybridization, 5-HT1A mRNA levels were also found to further increase in L1–L2 segments after a low-thoracic transection in 5-HT7-deficient mice compared with wild-type animals (Landry et al. 2006a). This was interpreted as evidence suggesting that even greater changes may be induced after trauma in the absence of closely related genes (i.e., 5-HT2). Results in paraplegic mice have also shown increased 5-HT2A mRNA levels in lumbar segments (laminae VII, VIII, and IX) several days after a complete low-thoracic transection (Ung et al. 2005).

Factors well recognized for their role in development and plasticity have also been found to be modulated post-trauma. Indeed, neurotrophic factors such as NGF, BDNF, and NT-3 were found to display up-regulated levels of expression in sublesional areas of T9/10 transected rats (Li et al. 2007). Because peak levels were observed at 7 and 14 days post-Tx, the authors have proposed a role for these neurotrophic factors in spontaneous locomotor function recovery in untrained and otherwise nonstimulated complete paraplegic animals. Their finding nicely supports those reported previously with immediate early genes in complete paraplegic mice (Landry et al. 2006b). In contrast, surprisingly, BDNF, NT-3, and synapsin-1 were found to display lower levels of expression in the lumbar cord of low-thoracic hemitranssected and deafferented rats (Gomez-Pinilla et al. 2004). This may suggest, as pointed out by the authors, that these decreasing levels were associated specifically with low levels of muscle activity due to deafferentation. Regular treadmill training was found to minimize the decreased expression, suggesting that training can modulate adaptive changes in sublesional networks (Ying et al. 2005).

In conclusion, this review has reported data describing some of the sublesional adaptive changes occurring spontaneously or induced by training within a few days to a few months after a complete transection of the spinal cord. Behavioral data suggest that CPG neurons may display increased excitability in complete paraplegic animals. Moreover, studies at the cellular level have revealed a number of changes involving, for instance, c-fos, nor-1, TNF-α, preprodynorphin, neurotrophic factors, and transmembranal receptors in areas of the spinal cord known to contain critical CPG elements. Although it has not yet been possible to directly associate these cellular changes with those at the behavioral level, there is increasing evidence suggesting that plasticity and cell property changes in neurons distally located from the epicenter (incomplete lesion or complete transection) may contribute to spontaneous and training-induced motor and locomotor function recovery. There is much to learn in this emerging new area but, hopefully, this field of research will continue to attract neuroscientists increasingly interested in spinal cord plasticity and functional recovery. Most would agree that this relatively new area of investigation in SCI research is likely to provide insights that will help in designing new therapeutic strategies for SCI patients.

REFERENCES


J Neurophysiol • VOL 98 • NOVEMBER 2007 • www.jn.org


