Intraspinal Stimulation Caudal to Spinal Cord Transections in Rats. Testing the Propriospinal Hypothesis

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Yakovenko S, Kowalczewski J, Prochazka A. Intraspinal stimulation caudal to spinal cord transections in rats. Testing the propriospinal hypothesis. J Neurophysiol 97: 2570–2574, 2007. First published January 10, 2007; doi:10.1152/jn.00814.2006. Many laboratories have reported the successful regeneration of neurons across damaged portions of the spinal cord. Associated improvements in hindlimb locomotor movements have been attributed to the formation of functional neuronal connections with the locomotor central pattern generator (CPG). However, regenerating axons generally extend no more than 10 mm caudal to the lesion sites, terminating about 20 mm short of the lumbar segments thought to contain the CPG. It has therefore tacitly been assumed that the locomotor improvements arose from activation of propriospinal neurons relaying excitation to the CPG. Here we report a test of this assumption, which we call the propriospinal hypothesis. Intraspinal microstimulation (ISMS) was used to activate the putative propriospinal relay neurons. Approximately 2–3 wk after complete spinal cord transection at T8–T9 in rats, an array of six Pt–Ir microwires was chronically implanted in the intermediate and ventral gray matter of T10–T12 segments. ISMS pulse trains with amplitudes of 0.8–0.9 times threshold for activating axial muscles were delivered during open-field locomotor tests (BBB). ISMS significantly increased BBB scores over control tests, but did not produce limb coordination and weight bearing sufficient for locomotion. These results support the main assumption of the propriospinal hypothesis: that neuronal activity elicited in thoracic spinal segments caudal to a complete spinal cord transection may propagate caudally and activate the locomotor CPG.

INTRODUCTION

Many recovery strategies are currently under development to restore spinal cord function after injury. Unlike peripheral nerves, damaged neurons in the CNS fail to regenerate, apparently because of the formation of impenetrable scar tissue (Fawcett and Asher 1999) and the presence of growth inhibitory molecules (Caroni and Schwab 1988; Schwab and Thoenen 1985). Aguayo and colleagues pioneered the use of peripheral nerve grafts to achieve long-distance axon regeneration in the CNS (Aguayo et al. 1981; Bray et al. 1987). Numerous types of graft and molecular strategies to promote regeneration have since been studied, particularly in rats. In some cases neuronal regeneration across complete spinal cord transections was associated with significant functional improvements, including the restoration of hindlimb weight-bearing and coordinated locomotion (Cheng et al. 1996; Cousins et al. 2001; Ramon-Cueto et al. 2000). These results are particularly crucial because completely spinalized rats do not recover weight-bearing locomotion spontaneously (Basso et al. 1996), although they may do so with extensive locomotor training (Edgerton et al. 2001). Another surprising feature of these results is that significant improvements were observed even though the extent of regeneration was quite limited. Typically the regenerated axons extended only one or two spinal segments below the lesions, which were usually in the mid- to lower thoracic segments (Bamber et al. 2001; Ramon-Cueto et al. 1998; Rapalino et al. 1998). In most cases this meant that the regenerating axons terminated about 20 mm short of the lumbar spinal cord containing the hindlimb locomotor pattern generator (Cazalets et al. 1995, 1998; Cowley and Schmidt 1997; Kjaerulff and Kiehn 1996).

What is the mechanism of the functional recovery accompanying the regeneration of axons below such lesions? One plausible explanation that is tacitly accepted in the regeneration field is that regenerating axons make connections with propriospinal neurons (PSNs), which extend caudally and activate hindlimb locomotor circuitry in the lumbar spinal cord (Fig. 1A). We now propose the term “propriospinal hypothesis” to describe this explanation. Recently Bareyre et al. (2004) found that injured corticospinal neurons sprouted and made functional connections with long PSNs projecting to the lumbar spinal cord. The propriospinal hypothesis would require that such connections are also made by transected axons growing through and beyond a complete spinal cord lesion (Fig. 1A). Furthermore, the hypothesis would require that nonspecific activation of these descending PSNs would activate elements of the central pattern generator (CPG) sufficiently to cause the observed locomotor improvements (Fig. 1B). The aim of our study was to test this latter part of the propriospinal hypothesis by nonspecifically activating PSNs with intraspinal microstimulation (ISMS) caudal to a complete spinal transection.

METHODS

Animals

The experiments were performed on 11 adult Sprague–Dawley female rats weighing 200–250 g. Animal treatment and surgical procedures were approved by the University of Alberta Health Sciences Animal Welfare Committee and conformed to the guidelines of the Canadian Council on Animal Care.

SPINAL CORD TRANSACTION Animals were deeply anesthetized with isoflurane and underwent an aseptic laminectomy at the level of
After postoperative recovery, we filmed and rated locomotor performance before and during ISMS using the standard Basso–Beattie–Bresnahan (BBB) open-field locomotor rating scale (Basso et al. 1995). The BBB test was chosen to allow comparisons between our results and those of many published regeneration studies. The bladder was manually emptied and motor thresholds of each of the implanted electrodes were determined 30 min before the experiment. Animals were rated in two successive 4-min sessions: a control session without ISMS and a test session with ISMS. The threshold of each microelectrode for eliciting contractions of trunk and abdominal muscles was individually determined at the start of each testing session. During the session, trains of stimulus pulses (biphasic, 200 μs, 50 s⁻¹) with amplitudes 0.8–0.9 times threshold (20–200 μA) were delivered through each microelectrode in the ISMS array in an interleaved sequence, such that the action of each microelectrode was independent of the others. The threshold of each electrode was determined by careful visual observation and palpation just before open-field testing sessions. The testing was conducted for 1–3 wk after implantation until the local motor thresholds exceeded 300 μA. The change in the threshold could have been caused by microelectrode migration over the course of several weeks or it could have been the result of electrode encapsulation, local tissue damage, or gradual failure of insulation at the connector.

Histology

At the end of the experiment animals were deeply anesthetized with sodium pentobarbital and perfused through the heart with a 3.7% formaldehyde solution. Thoracic and lumbar spinal columns were extracted and the positioning of the arrays within the T10–T11 spinal segments was confirmed using thoracic vertebrae as reference. We extracted and the positioning of the arrays within the T10–T11 spinal segments was confirmed using thoracic vertebrae as reference. We were only partly successful in histologically identifying microelectrode tip positions in the chronically implanted animals. The extensive growth of connective tissue in the area of the spinal cord injury extended to the site of implantation and provided a challenge to remove without dislocating the microwires. In four animals, the thoracic segments of the spinal cord with embedded microelectrodes were manually sectioned and the relative positions of the implanted microelectrode tips were established to confirm the accuracy of targeting during implantation.

Statistics

To compare changes in the BBB scores in response to the microstimulation, the difference in scores before and during stimulation is reported as mean ± SD. The critical significance level α was set at 0.05. A result was considered significant if the achieved significance P value was lower than α. Bootstrap analysis was used as an additional nonparametric test with the same α. Bootstrapping is a procedure for estimating the distribution of a data set by resampling with replacement from the original sample. The variation of the resulting difference between the scores was achieved by comparing the difference between randomly chosen data samples 10,000 times. The confidence interval was then calculated as 2.5 and 97.5 percentiles of the resulting distribution. This method is effective for testing mediation for small samples of data without the requirement for the normality assumption to be met.

RESULTS

To test the “propriospinal hypothesis” rats were implanted with ISMS arrays below a T8 lesion, ±10 mm rostral to the L1 spinal segment. Figure 2A shows the relative position of the tips of the implanted electrodes, which targeted intermediate and ventral gray matter. Figure 2B shows
not rely on the assumption of a normal distribution (Efron and Tibshirani 1993). Figure 3C shows a resampled population of the differences of BBB scores between the control and test conditions. The median difference of 1.5 (solid gray line) remained significant for 95% confidence interval [0.4, 2.7] (dashed gray lines).

It is important to note that, although the change in the motor performance was very small, it was comparable to the changes reported in numerous regeneration studies. Furthermore, a difference of 1.5 on the BBB scale represents an almost 50% increase on the baseline regeneration score. This indicates that nonspecific electrical activation of T10–T11 spinal segments can modestly improve locomotor performance of rats with complete spinal transections assessed by the BBB open-field locomotor rating scale.

**DISCUSSION**

This study demonstrates that tonic ISMS of gray matter immediately caudal to a complete spinal cord transection and several segments rostral to the region assumed to contain the locomotor CPG improves locomotor performance in adult rats. The extent of the improvement scored according to the BBB open-field locomotor scale was comparable to that after long-distance axon regeneration below the site of a spinal cord injury (GrandPre et al. 2002; Hausmann et al. 2002; McDonald et al. 1999; Tuszynski et al. 2003; Verdu et al. 2003). Overall,
these results support an important component of the proprio-
spinal hypothesis: that nonspecific activation of descending
PSNs may partly activate the locomotor CPG.

PSNs are located in ventral as well as in dorsal laminae of
the spinal cord and in fact are likely to represent the majority
of spinal neurons (Chung et al. 1984, 1987; Menetrey et al.
1985; Skinner et al. 1979). Midthoracic PSNs are likely to be
involved in coordinating the activity of the cervical and lumbar
enlargements and thus mediating forelimb–hindlimb coupling
(Juvin et al. 2005). Our choice of intermediate and ventral
areas as targets for stimulation was based on the evidence that
sparing of the gray matter in these areas after spinal cord injury
is more correlated to locomotor recovery than sparing of dorsal
laminae (Schucht et al. 2002; You et al. 2003). However, this
does not rule out the potential importance of dorsal proprio-
spinal pathways, which may also be involved in the activation of
the hindlimb locomotor CPG. This is supported by the
evidence of locomotor recovery induced by epidural stimula-
tion of the most caudal thoracic and lumbar segments below a
spinal cord lesion (Gerasimenko et al. 2003; Ichiyama et al.
2005).

Three alternative possibilities should be mentioned. The first
is that ISMS (or axons regenerating through grafts) could
activate neurons that elicit contractions in local trunk muscles.
These contractions could stretch hip muscles and evoke pro-
proprioceptive feedback to the locomotor CPG (Giszter et al.
1998). This in turn could improve performance in the open-
field locomotor tests, without any direct activation of descend-
ring PSNs. Although we cannot eliminate this mechanism, it
does not satisfactorily explain improvements we observed in
foot and toe movements in the absence of improvements at the
hip.

The second possibility is that we may have activated sensory
axons, which have a low threshold to ISMS (Gaunt et al. 2006).
Thus we may have antidromically activated terminal branches
of group I and group II afferents, which project rostrally and
caudally from their entry points in the dorsal columns to
provide excitatory input to motoneurons up to two segments
away (Henneman and Mendell 1981). We doubt this for two
reasons. First, all afferent projections descending from dorsal
roots T8 and above were severed by the T8 transection.
Second, the stimulated areas were well within the range of
secondary spinal cord injury, where remaining axonal path-
ways go through a process of extensive demyelination and
retraction (Beattie et al. 2000). It is therefore unlikely that we
were antidromically stimulating afferents of more caudal tho-
racic or even lumbar segments, although again the possibility
cannot be entirely ruled out.

The third possibility is that neuronal networks in the caudal
part of the thoracic spinal cord, which were previously shown
to be capable of generating rhythmic activity in neonatal rats
(Cowley and Schmidt 1997) are in fact part of the hindlimb
locomotor pattern generator. ISMS of thoracic oscillators could
coneivably generate rhythmical waves of excitation propa-
gated caudally, as seen in lower vertebrates (Matsushima and
Grillner 1992). Neonatal rats are capable of locomotion and
rhythmical activity of axial trunk muscles is correlated with
that of hindlimbs during gait at moderate speeds. However, as
rats mature, the correlation disappears (Gramsbergen et al.
1999) except in high-speed locomotion, where postural stabi-
zation is needed and stretch reflexes are significant (Macpher-
son and Fung 1998; Zedka and Prochazka 1997; Zomlefer et al.
1984). Thus it is unlikely that thoracic and lumbar spinal cord constitute a common locomotor pattern generator, which

The limited amount of functional improvement in our ISMS
trials points to a possible limitation of recovery strategies based
only on a nonspecific activation of thoracic descending PSN
systems. In our experiments, none of the animals before or
during ISMS developed sufficient weight-bearing or intra- and
interlimb coordination necessary for locomotion. However, the
spared spinal cord was capable of a high level of coordination
and rhythmogenesis as was evident from long-lasting bouts of
coordinated air-stepping observed after mechanical stimulation
applied during bladder expression in the same animals. This
observation is in agreement with the recent finding of pathways
from sacrocaudal afferents to the lumbar sacral locomotor pat-
ttern generator in neonatal rats (Strauss and Lev-Tov 2003).
Also, it suggests the possibility that PSNs may increase loco-
motor performance not by the direct activation of lumbar
locomotor CPG networks, but by a nonspecific increase in the
overall motoneuron excitability. It is possible that the efficacy
of descending thoracic PSN inputs could be potentiated when
combined with other recovery strategies, e.g., locomotor train-
ing, activation of the lumbar sacral spinal cord with ISMS,
epidural stimulation, and/or pharmacological agents. Finally,
these results point out the necessity to identify the specific
descending propriospinal pathways that are involved in medi-
ating the observed improvement. This future direction of re-
search may have an important bearing on the success of studies
promoting regeneration in the spinal cord.

To conclude, in this study we tested a key part of “the
propriospinal hypothesis”: that nonspecific activation of de-
sceding pathways below a complete midthoracic transection
can improve locomotor performance. We found that tonic
ISMS of the gray matter immediately below the lesion pro-
duced small, but significant improvements of locomotor per-
formance comparable to those observed in studies of long-
distance axon regeneration.

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References

Aguayo AJ, David S, Bray GM. Influences of the glial environment on the
evolution of axons after injury: transplantation studies in adult rodents. J

Bamber NI, Li H, Lu X, Oudega M, Aebscher P, Xu XM. Neurotrophins
BDNF and NT-3 promote axonal re-entry into the distal host spinal cord
through Schwann cell-seeded mini-channels. Eur J Neurosci 13: 257–268,

Bareyre FM, Kerschensteiner M, Raineteau O, Mettenleiter TC, Wein-
mann O, Schwab ME. The injured spinal cord spontaneously forms a new

Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor

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