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Electrophysiological characterization of spontaneous recovery in deep dorsal horn interneurons after incomplete spinal cord injury

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ABSTRACT

In the weeks and months following an incomplete spinal cord injury (SCI) significant spontaneous recovery of function occurs in the absence of any applied therapeutic intervention. The anatomical correlates of this spontaneous plasticity are well characterized, however, the functional changes that occur in spinal cord interneurons after injury are poorly understood. Here we use a T10 hemisection model of SCI in adult mice (9–10 wks old) combined with whole-cell patch clamp electrophysiology and a horizontal spinal cord slice preparation to examine changes in intrinsic membrane and synaptic properties of deep dorsal horn (DDH) interneurons. We made these measurements during short-term (4 wks) and long-term (10 wks) spontaneous recovery after SCI. Several important intrinsic membrane properties are altered in the short-term, but recover to values resembling those of uninjured controls in the longer term. AP discharge patterns are reorganized at both short-term and long-term recovery time points. This is matched by reorganization in the expression of voltage-activated potassium and calcium subthreshold-currents that shape AP discharge. Excitatory synaptic inputs onto DDH interneurons are significantly restructured in long-term SCI mice. Plots of sEPSC peak amplitude vs. rise times suggest considerable dendritic expansion or synaptic reorganization occurs especially during long-term recovery from SCI. Connectivity between descending dorsal column pathways and DDH interneurons is reduced in the short-term, but amplified in long-term recovery. Our results suggest considerable plasticity in both intrinsic and synaptic mechanisms occurs spontaneously in DDH interneurons following SCI and takes a minimum of 10 wks after the initial injury to stabilize.

1. Introduction

An incomplete spinal cord injury (SCI) disrupts axonal pathways and spinal cord circuitry to produce severe motor, sensory and autonomic defects. Regardless of the degree of injury it appears that some spontaneous recovery of function occurs after incomplete SCI. For example, work in the 1920s showed cats exhibited significant recovery of hindlimb function following spinal hemisection (Pike et al., 1929). Later experiments showed that coordinated locomotor responses abolished by an SCI could return, even in cases where as little as 5–10% of spinal axons were spared (Blight, 1983; Eidelberg et al., 1977; Windle et al., 1958). It is now accepted that improvement in function can only begin after the resolution of spinal shock and the metabolic chaos and inflammation that accompanies acute SCI (Alstermark et al., 1987; Jeffery and Blakemore, 1999). Such functional recovery begins ~3–4 weeks after the SCI (Alstermark et al., 1987; Courtine et al., 2008; Eidelberg et al., 1981; Helgren and Goldberger, 1993; Kato, 1989; Little et al., 1988).

Spontaneous recovery occurs in the absence of any applied intervention, such as exercise training or other therapies, and much effort has been invested in understanding the mechanisms involved. It is now well accepted that changes in spinal cord circuitry, both rostral and caudal to an injury, as well as changes in supraspinal structures underpin spontaneous recovery (Beattie et al., 1997; Fenrich and Rose, 2009, 2011; Fouad et al., 2001; Hill et al., 2001; Lawrence and Kuypers, 1968; Oudega and Perez, 2012; Weidner et al., 2001). Recent experiments using a dual lesion model of SCI in cats have shown that recovery of hindlimb locomotion depends on plasticity in spared descending pathways, and within spinal circuits caudal to the lesion (Barriere et al., 2008; Frigon, 2009; Martinez et al., 2011). Further, anatomical studies in rodent models of incomplete SCI have shown that both intact and damaged axons in the vicinity of a spinal lesion sprout to form new intraspinal circuits (Bareyre et al., 2004; Beattie et al., 1997; Courtine et al., 2008; Fouad et al., 2001; Goldshmit et al., 2008; Goldstein et al.,

Abbreviations: AHP, after hyperpolarization; AP, action potential; ACSF, artificial cerebrospinal fluid; CC, contralateral caudal; CE, contralateral epicenter; CR, contralateral rostral; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DC, dorsal column; DDH, deep dorsal horn; DF, delayed firing; ES, effect size; I_{Ar}, rapid I_A current; I_{As}, slow I_A current; IB, initial bursting; IC, ipsilateral caudal; I_{Ca}, T-type calcium current; IR, ipsilateral rostral; R_{in}, input resistance; RMP, resting membrane potential; SCI, spinal cord injury; sEPSC, spontaneous excitatory postsynaptic potential; SS, single spiking; TF, tonic firing.

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1997; Onifer et al., 2011; Rank et al., 2015). Most importantly, these new sprouts have the capacity to reconnect with their originally denervated targets (Bareyre et al., 2004; Courtine et al., 2008; Fenrich and Rose, 2009; Fouad et al., 2001). Notably, the time course of anatomical plasticity parallels the spontaneous recovery of function that occurs in the weeks and months following SCI (Bareyre et al., 2004; Courtine et al., 2008; Flynn et al., 2011a,b, 2013; Fouad et al., 2001; Goldshmit et al., 2008; Goldstein et al., 1997; Onifer et al., 2011). Thus, the available data suggest that spontaneous recovery of function in animal models of incomplete SCI occurs within pre-existing and newly formed circuits via anatomical reorganization of axons and their synapses (reviewed in Raineteau and Schwab, 2001).

While considerable data on anatomical reorganization (i.e. plasticity) following SCI are available, fewer studies explore the functional changes that occur in spinal cord neurons during spontaneous recovery. Most of the available functional data describe changes to motoneurons, especially those involved in the generation of rhythmic locomotor movements in the hindlimb musculature of quadrupedal mammals (Barbeau and Rossignol, 1987; Beaumont and Gardiner, 2002, 2003; Beaumont et al., 2008; Lovely et al., 1990; Petruska et al., 2007). Much less is known about the functional properties of other neuron populations, such as interneurons, within the spinal cord during spontaneous recovery from SCI. This is surprising as descending inputs, which ultimately drive motor behaviors, often arrive at motoneurons via interposed interneurons. Axons of the corticospinal tract, for example, terminate in the deep dorsal horn (DDH) of the spinal cord in primates, cats, and rodents (Galea and Darian-Smith, 1994; Tracey, 2004). In rodents, the only connection between corticospinal axons and motoneurons is via DDH interneurons. Thus, DDH interneurons are positioned to receive descending inputs and recent work suggests they are ideally placed to "bridge a lesion" by forming new functional intraspinal circuits (Bareyre et al., 2004; Courtine et al., 2008; Fenrich and Rose, 2009; Fouad et al., 2001).

We have previously reported, using whole-cell patch-clamp electrophysiology and an adult mouse hemisection model of SCI, that DDH interneurons exhibit considerable synaptic plasticity following 3 and 6 wks of treadmill exercise training (Flynn et al., 2013; Rank et al., 2015). These data provide additional support for the enhanced formation of functional de novo intraspinal circuits in DDH interneurons following incomplete SCI, and specifically a role for exercise training as an intervention to improve recovery. However, we know very little about *spontaneous* functional recovery in these DDH interneurons nor its time course. Accordingly, we have examined the intrinsic membrane and synaptic properties of DDH interneurons during *spontaneous recovery* at 4 weeks (i.e. short-term) and 10 weeks (i.e. long-term) after SCI and compared these properties to those in uninjured age-matched animals. By comparing these data to uninjured control animals we can gain an important perspective on how the spontaneous plasticity exhibited by DDH interneurons after incomplete SCI compares to their properties in the uninjured spinal cord.

2. Materials and methods

Approval for all procedures was obtained from The University of Newcastle Animal Care and Ethics Committee (Approval # A-2009-154) according to the NSW Animal Research Act, 1985. Some of the procedures for the SCI surgery, data collection and analyses have been described previously (Flynn et al., 2011a, 2013; Rank et al., 2015), and are only briefly summarized here.

2.1. Hemisection surgery

Adult male C57/BL6 mice (aged 9–10 wks) received a left T10 spinal cord hemisection (between T10 and T11 spinal nerves) under isoflurane anesthesia. Buprenorphine (0.1 mg/kg s.c., every 8 h for 2 d) and carprofen (5 mg/kg s.c, every 24 h for 5 d) were administered for post surgical analgesia. The same investigator performed all surgeries. Following surgery, animals were returned to their home cages and were allowed to recover. Animals were housed two to three per cage (measuring 34 cm × 16 cm × 12 cm) with standard cage furnishings (corn bedding, domed hide, cardboard tube) and ad lib access to food and water. In all mice, the ipsilateral/left hindlimb was paralyzed and non-functional immediately following surgery. Gradual spontaneous improvements in function of the left hindlimb occurred in the weeks following SCI. It is important to note, however, that as the mice in our study received an incomplete/lateral hemisection SCI, they retained near normal movement in the contralateral/unaffected hindlimb. This means the animals were able to move around the home cage and engage in other normal cage activities soon after surgery.

To control for lesion variability we quantified the lesion area and lesion extent (mean ± SEM) in spinal cord slices used for recording (see dashed rectangle in Fig. 1B, C). Lesion area was simply the area encompassed by the wedged-shaped wound in spinal cord slices. Lesion area was similar in short- and long-term SCI mice (0.53 ± 0.08 vs 0.40 ± 0.06 mm^2; $p = 0.18$). We also measured lesion extent, which we defined as the distance between the medial apex of the lesion and the midline of the spinal cord. In this analysis a value of 0 mm indicates that the lesion apex was at the midline of the cord. Lesion extent was similar in short- and long- term SCI mice (0.15 ± 0.03 vs 0.09 ± 0.02 mm; $p = 0.12$). Together, these data suggest that our SCI hemisection lesions were similar in all mice. We did not quantify the lateral extent of the SCI. As evident in Fig. 1, no recordings were obtained from neurons immediately adjacent to the hemisection.

After surgery, animals that recovered for 4 wks are hereafter called *short-term* SCI mice (aged 13 wks at time of terminal in vitro electrophysiological experiments) and animals that recovered for 10 wks are called *long-term* SCI mice (aged 19 wks at time of terminal experiment). Uninjured animals were age-matched to the short- and long-term SCI groups (13 or 19 wks old), and are hereafter termed controls. Experiments were designed with separate control groups for the short- and long-term SCI mice. Statistical comparisons made after the data were collected revealed no differences between the 13 and 19 wk old control groups therefore we pooled these data to form a single uninjured control group for comparisons.

2.2. Tissue preparation

Under deep ketamine anesthesia (100 mg/kg i.p.) animals were decapitated and horizontal spinal cord slices were prepared as previously described in Flynn et al., 2011a. Briefly, the mouse torso was submerged in ice-cold, oxygenated, sucrose substituted artificial cerebrospinal fluid (sACSF containing (in mM): 250 sucrose, 25 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 6 $MgCl_2$, and 1 $CaCl_2$; pH 7.3) and a length of spinal cord (T5–T13) was removed. The spinal cord was mounted on an agar block (6% agarose) using cyanoacrylate glue (Loctite 401, Loctite, Caringbah, Australia). Horizontal spinal cord slices (250 μm thick) were cut using a vibrating microtome (HM 650 V; Microm, Walldorf, Germany or VT1000s, Leica Microsystems, Nuslock, Germany). A horizontal slice containing the deep dorsal horn (including laminae IV and V; Fig. 1A) and a continuous strip of the dorsal columns (DCs) was selected and transferred to a recording bath for electrophysiology. Slices were perfused with oxygenated ACSF (containing (in mM): 118 NaCl, 25 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4 and 1 $MgCl_2$ and 2.5 $CaCl_2$). The spinal cord slice was secured in the bath, by a custom-made platinum and nylon net, and allowed to equilibrate for 30–45 min at room temperature (22–25 °C) before commencing experiments. A bipolar stimulating electrode was placed in the dorsal columns rostral to the transection to study dorsal column evoked synaptic responses (Fig. 1).