THE EFFECTS OF AXOTOMY ON THE BIOPHYSICAL PROPERTIES OF RETICULOSPINAL NEURONS IN LARVAL LAMPREY

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THE EFFECTS OF AXOTOMY ON THE BIOPHYSICAL PROPERTIES OF RETICULOSPINAL NEURONS IN LARVAL LAMPREY

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ABSTRACT

Immediately after severe spinal cord injury (SCI), animals are paralyzed below the lesion. The degree of recovery following SCI depends on both phylogeny and ontogeny. Adult higher vertebrates, such as birds and mammals, exhibit little to no axonal regeneration, and there is minimal recovery of function (Schwab and Bartholdi, 1996). In contrast, following SCI in lower vertebrates, such as lamprey, descending brain neurons, including RS neurons, regenerate their axons, and locomotor function recovers in a few weeks (McClellan, 1992, 1994, 1998; also see Rovainen, 1976), even though axonal regeneration is incomplete.

Following SCI in larval lamprey, RS neurons undergo a number of changes in their biophysical properties (McClellan et al. 2002; McClellan, 2003). These changes might simply be a reaction to injury or may represent potentially important cellular or molecular changes that are critical for subsequent repair and regeneration. Following axotomy of large, identified RS neurons (Müller cells) in the lamprey, most studies have examined morphological or molecular changes in cell properties. Previously in our laboratory, it was shown that a rostral spinal cord transection is a much stronger stimulus for axonal regeneration than a caudal transection (McClellan et al., 2005). Furthermore, locomotor muscle activity recovers just below a rostral spinal transection after 2-3 weeks, while recovery of muscle activity below a caudal spinal transection takes much longer. Finally, following rostral spinal cord transections, the axons of most RS neurons regenerate for relatively short distances and presumably make synapses just below the lesion (Davis and McClellan, 1994a,b). Together, these data suggest the hypothesis that the degree to which a neuron is stimulated to regenerate is dependent, in part, on synaptic
connections it makes, above and/or below a spinal cord lesion (McClellan, 1998). Based on the above information, we hypothesized that rostral spinal cord transections will alter the biophysical properties of axotomized RS neurons to a greater degree than more caudal transections.

Following right hemi-transections in the rostral spinal cord, the majority of RS neurons with injured descending axons displayed a number of properties typical of injury. In contrast, following right hemi-transections in the caudal spinal cord, the majority of RS neurons with injured descending axons displayed properties typical of normal, uninjured RS neurons. At a given recovery time, RS neurons with rostral hemi-transections had significantly larger fast (fAHP) and significantly smaller slow (sAHP) afterhyperpolarizations compared to RS neurons with caudal hemi-transections or to uninjured RS neurons. Furthermore, following rostral right hemi-transections and 2 week recovery times, RS neurons that displayed bursting or high spike frequency adaptation (SFA) firing patterns had significantly larger fAHPs and significantly smaller sAHPs than neurons that displayed smooth, regular firing patterns. Therefore, the present study suggests that rostral spinal cord transections are a stronger stimulus not only for axonal regeneration but also for changes in biophysical properties than caudal transections.

At least some of these changes in biophysical properties may be important in allowing RS neurons in the lamprey to regenerate their axons. For example, sAHP are largely due to calcium influx and calcium-activated potassium channels, and a reduction in this component of the AHP in injured RS neurons may be due to down regulation of calcium channels. Also, cell culture experiments in our laboratory suggest that relatively low intracellular levels of calcium are necessary for neurite outgrowth. Thus, injured RS
neurons may down regulate calcium channels to reduce intracellular calcium levels and to promote axonal regeneration. If the specific changes in biophysical and molecular properties of RS neurons that promote axonal regeneration can be identified in the lamprey, the information may be helpful in understanding why neurons in higher vertebrates, including humans, do not regenerate following spinal cord injury.
INTRODUCTION

General Organization of Locomotor System

In vertebrate animals, for both anatomical and physiological reasons, the neural networks that produce locomotor behavior can be divided into at least three components (reviewed by Stein 1978; Grillner 1981; McClellan 1986, 1996). Pattern generators consist of oscillators or local control centers that are distributed along the spinal cord. A spinal coordinating system couples the oscillators to coordinate the locomotor activity for different regions of the body. Together, the pattern generators and coordinating system constitute a central pattern generator (CPG) that can generate the basic motor patterns for locomotion in the absence of sensory feedback, but sensory inputs are critical in shaping or “fine tuning” locomotor patterns. Command systems in the brain activate the spinal CPGs to initiate locomotor behavior. Reticulospinal neurons (RS) are the output neural elements of the command system that receive inputs from higher order locomotor areas in the brain and that project descending axons directly to the spinal cord to initiate locomotor behavior. The locomotor systems have a similar basic organization in a wide variety of vertebrates (reviewed by Stein 1978; Grillner 1981; McClellan 1986, 1996).

Spinal Cord Injury

Following severe spinal cord injury (SCI), the axons of RS neurons are disrupted, and initially animals are paralyzed below the lesion (McClellan, 1998). The degree of recovery following SCI depends on both phylogeny and ontogeny. Adult higher vertebrates, such as birds and mammals, exhibit little to no axonal regeneration, and there is very little recovery of function (Schwab and Bartholdi, 1996). In embryonic or
immature higher vertebrates, prior to myelination, injured axons do regenerate, and there is substantial recovery of motor functions below the lesion (Steeves et al., 1994). In contrast, following SCI in lower vertebrates, such as lamprey, fish, and certain amphibians, injured axons display robust regeneration and synaptic specificity with targets below the lesion so that recovery of behavioral activities, such as locomotion, is virtually complete (McClellan, 1998).

Following spinal cord transection in larval lamprey, a lower vertebrate, descending brain neurons, including RS neurons, regenerate their axons and locomotor function eventually recovers (McClellan, 1992, 1994; also see Rovainen, 1976). However, axonal regeneration is incomplete. Specifically, in normal lamprey, the majority of RS neurons project for long distances to all levels of the spinal cord. After spinal cord transections and axonal regeneration, the axons of most RS neurons regenerate through the injury site and appear to make synapses just below the lesion site (Davis and McClellan, 1994a,b; also see Rovainen, 1976). Relatively few of the axons of RS neurons regenerate for long distances.

**General Introduction to Axotomy**

Axotomy, the disruption of axons, causes a number of neurophysiological, anatomical, and molecular changes in injured neurons and surroundings tissue. For example, axotomy can cause changes in several biophysical properties, such as resting membrane potential, amplitude and duration of action potentials, firing properties, and conduction velocity (Titmus and Faber, 1990). Within the nervous system, neurons can have a wide variety of functions, morphologies, and synaptic connections, as well as variability in response to injury. Not only do the responses to axotomy vary between
neuron types, such as motoneurons versus sensory neurons, but also the responses of the same type of neuron vary in different organisms.

For sensory neurons, following axotomy of trigeminal sensory neurons in mouse, resting membrane potential, spike threshold, and input resistance decrease, but action potential amplitude or duration do not change (Cherkas et al., 2003). Axotomy of sensory neurons in mouse dorsal root ganglia (DRG) result in an increase in resting membrane potential, the fast component of the afterhyperpolarization (fAHP), and duration of the AHP, and a decrease in action potential amplitude, but action potential duration remains unchanged (Liu et al., 2002). However, following axotomy of sensory neurons in rat DRG in different studies, a wide variety of changes in biophysical properties were noted: (a) resting membrane potential increases (Flake et al., 2004), decreases (Liu et al., 2000), or does not change (Abdulla and Smith, 2001; Stebbing et al., 1999; Zhang et al., 1997); (b) threshold decreases (Liu et al., 2000; Abdulla and Smith, 2001; Zhang et al., 1997); (c) action potential amplitude increases (Liu et al., 2000; Abdulla and Smith, 2001), decreases (Flake et al., 2004), or does not change (Stebbing et al., 1999); (d) duration of the action potential increases (Liu et al., 2000; Abdulla and Smith, 2001; Flake et al., 2004; Kim et al., 1998; Stebbing et al., 1999) or does not change (Zhang et al., 1997); and (e) input resistance increases (Flake et al., 2004) or decreases (Kim et al., 1998).

For motoneurons, similar changes have been reported for axotomized cat motoneurons: (a) increases in input resistance (Pinter et al., 1991; Pinter and Noven, 1989; Foehring et al., 1986; Gustafsson, 1979; Yamuy et al., 1992); (b) increases in AHP duration (Pinter et al., 1991; Pinter and Noven, 1989; Yamuy et al., 1992); and (c)
decreases in conduction velocity (Pinter et al., 1991; Pinter and Noven, 1989; Foehring et al., 1986; Yamuy et al., 1992; Nishimura et al., 1992; Milner and Stein, 1981; Mendell et al., 1976). However, some differences have been reported for different types of motoneurons. Cat medial gastrocnemius (soleus) motoneurons display no change (a decrease) in the resting membrane potential, no change (increase) in input resistance, a decrease (no change) in the AHP duration, and a decrease (decrease) in the conduction velocity (Kuno et al., 1974). Axotomy of sympathetic neurons in the bullfrog results in an increase in action potential amplitude (Gordon et al., 1987; Kelly et al., 1988) and duration (Gordon et al., 1987; Shapiro et al., 1987), a decrease in the amplitude of the fAHP (Kelly et al., 1988; Shapiro et al., 1987; Smith et al., 1986), sAHP (Gordon et al., 1987; Kelly et al., 1988), and a decrease in AHP duration (Gordon et al., 1987; Kelly et al., 1988; Smith et al., 1986). In contrast, axotomy of rat sympathetic neurons results in a decrease in action potential amplitude (Sanchez-Vives and Gallego, 1993). Axotomy of interneurons in the central nervous system (CNS) of rats seems to produce changes in biophysical properties that are less variable than those following axonal injury in the peripheral nervous system (PNS). For example, in several studies in which descending brain neurons (i.e. corticospinal or rubrospinal) were injured in rat, the results were relatively consistent: (a) no changes in resting membrane potential (Tseng and Price, 1996; Mandolesi et al., 2004; Chen and Tseng, 1997), action potential amplitude (Tseng and Price, 1996; Chen and Tseng, 1997), or action potential duration (Tseng and Price, 1996; Chen and Tseng, 1997); and (b) an increase in input resistance (Tseng and Price, 1996).
Even for the same neuron type and model organism, some similarities and some differences in response to injury have been reported. For example, in the goldfish Mauthner cell, a large, identified RS neuron involved in initiating escape responses, two different studies found that axotomy results in no change in resting membrane potential and an increase in action potential amplitude. In contrast, one study found that input resistance increases (Faber and Zottoli, 1981) while another study found no change (Titmus et al., 1986). These different results might be due to differences in techniques or sample size, either of which may or may not explain some of the differences in the results in other studies.

The changes in biophysical properties that occur following axotomy might simply be a reaction to injury or potentially linked to initial cellular or molecular changes that are important for subsequent repair and regeneration. Following axotomy, mammalian CNS neurons are thought to pass through three phases, including an injury phase, a sprouting phase, and arrested axonal growth at the lesion scar (Abankwa et al., 2002). Abankwa et al (2002) found changes in gene expression of 125 genes in injured rat projection neurons as they pass through the above three phases. In rat motoneurons, protein synthesis and glucose utilization increase during the first 21 days post-axotomy and then return to normal between 24-35 days (Smith et al., 1984). Following axotomy of rat facial motoneurons, synthesis of tubulin and actin increases, while synthesis of neurofilaments decreases (Tetzlaff et al., 1988). Anterograde transport of endogenous brain derived neurotrophic factor (BDNF) in injured rat DRG neurons increases 3.5-fold within 24 hours post-injury of the sciatic nerve, which suggests that BDNF plays a role in early response to peripheral nerve injury (Tonra et al, 1998). Additionally, after sciatic
nerve injury, mRNA encoding three integrins are up-regulated in rat DRG neurons, which are able to regenerate their peripheral axons, suggesting that integrins may have important roles in the regenerative response (Wallquist et al., 2004). After axotomy of buccal neurons in Aplysia, a marine mollusk, intracellular calcium concentrations are elevated above the physiological range near the tip of the cut axon but recover to control levels within 2-3 minutes after resealing (Ziv and Spira, 1995). Perhaps intracellular changes, such as a large influx of calcium, begin a cascade of events that lead to regenerative processes. For example, CaM kinase IIa increases following axotomy of rat motoneurons and appears to support axonal growth (Lund and McQuarrie, 1997). Following lesions of peripheral nerves, growth-associated protein 43 (GAP-43) mRNA is up regulated in both ipsilateral, axotomized motoneurons and contralateral, uninjured motoneurons in the rat, cat, and monkey (Linda et al., 1992). Furthermore, following permanent axotomy of cat motoneurons, GAP-43 is immunoreactive in axon-like dendrite processes (Rose et al., 2001).

Following axotomy in the CNS, anatomical changes are also evident in injured neurons. Just as there is a variation in electrophysiological and molecular responses to axotomy, there is also variation in anatomical responses to injury. Following axotomy of different CNS neurons, a number of morphological changes occur: (a) the dendritic tree increases (Mandolesi et al., 2004), decreases (Tseng and Hu, 1996), or does not change (Tseng and Prince, 1996); and (b) soma diameter increases (Faber and Zottoli, 1981; Wood and Faber, 1986) or decreases (Pastor et al., 2000; Tseng and Prince, 1996; Tseng and Hu, 1996). After axotomy of rat rubrospinal neurons, the soma size increases at 3-4 days post-axotomy, but then decreases during 4-10 weeks post-axotomy (Chen and
Tseng, 1997). Other changes in the soma of axotomized Mauthner cells in goldfish include chromatolysis, inward folding of the nuclear membrane, and basophilic material accumulation (Faber and Zottoli, 1981), as well as a decrease in density of somatic synaptic terminals (Wood and Faber, 1986). In contrast, following axotomy of cat abducens internuclear neurons, Pastor et al. (2000) report no change in cytological features.

Similarly, following peripheral axotomy of motoneurons, variable changes in morphology have been reported: (a) the dendritic tree increases (Rose and Odlozinski, 1998) or decreases (Brannstrom et al., 1992; Linda et al., 1992); and (b) the soma diameter increases (Linda et al., 1992), decreases (Umemiya et al., 1993) or does not change (Nishimura et al., 1992). Following permanent axotomy of cat motoneurons, the soma diameter initially increases by 3 weeks post-injury, and then gradually returns to normal by 12 weeks (Brannstrom et al., 1992).

Summary of Effects of Axotomy in Lamprey Neurons

In the lamprey, some anatomical, molecular, and electrophysiological changes in brain and spinal cord neurons following axotomy have been described. Certain anatomical changes resulting from axotomy of dorsal cells (i.e. centrally located sensory spinal neurons), such as an increase in nuclear diameter and decrease in cell diameter, are apparent when axotomy is <30 mm from cell bodies (Yin et al., 1981). For large, identified Müller cells (i.e. RS neurons), proximal axotomy <500 µm from the cell body elicits predominantly dendritic sprouting, whereas distal axotomy 15-20 mm from cell bodies evokes predominantly axonal sprouting with virtually no dendritic sprouting (Hall and Cohen, 1983, 1988, 1988). The net amount of sprouting, however, is not affected by
the location of axotomy, and the dendritic and axonal sprouts have similar morphological characteristics and rostro-caudal trajectories. Eventually, following axotomy close to Müller cell bodies, all dendritic sprouts retract, as does the original dendritic tree. Anatomically, during metamorphosis of larval lamprey to the adult phase, there is little change in either the size or the dendritic morphology of large, identified RS neurons (Swain et al, 1995). For smaller RS neurons, the distance of descending projections increases during metamorphosis with little increase in the total number of RS neurons. Following axotomy (i.e. spinal cord transection) at 25% body length (BL, relative distance from the head) performed during metamorphosis, there is an increase of sprouting in the dendrites of RS neurons, an increase in the efficiency of retrograde transport of anatomical tracers, and increase of axonal dieback (Swain et al, 1995).

Following a rostral spinal cord transection, large, identified RS neurons display a number of molecular changes, including increases in NF phosphorylation and tubulin tyrosination prior to the emergence of sprouts from dendrites, in which acetylated tubulin is reduced (Hall et al., 1991). At the time of axotomy, PKC/ PKA inhibitors block somatodendritic NF phosphorylation. Conversely, in the absence of axotomy, injection of PKA catalytic subunits induces somatodendritic NF phosphorylation (Hall and Kosik, 1993). For the axons of Mauthner cells, rostral axotomy results in sidearm proteolysis in axonal NFs, leading to the rod domain staining seen in the dense mass in the retraction bulb in degenerating axons (Hall and Lee, 1995). With the onset of regeneration, this staining disperses, indicating that the presence or absence of staining is correlated with degenerative or regenerative processes.
Following axotomy, dorsal cells (DCs) in the spinal cord of lamprey display a number of altered electrophysiological properties (Yin et al., 1981). Resting membrane potential decrease 5 weeks post axotomy, but increase by 11 wks. Also, there is a decrease in maximal rate of rise of action potentials. Voltage and current thresholds, action potential amplitude and spike width, input resistance, conduction velocity and spike overshoot increase following axotomy. In contrast to the anatomical effects described above, there was no clear relationship between electrophysiological changes and the proximity of the cell body to the transection site. Furthermore, the anatomical changes were maximal at 3 weeks post axotomy, while the electrophysiological changes were maximal at 4-5 weeks.

**Specific Background and Rationale for the Present Study**

Following axotomy of large, identified RS neurons (Müller cells) in the lamprey, most studies have examined morphological or molecular changes in cell properties. In our laboratory we have demonstrated that following SCI in larval lamprey, RS neurons undergo a number of changes in their biophysical properties (McClellan et al. 2002; McClellan, 2003). At one to three weeks following a hemi-spinal cord transection at 10% BL, axotomized RS neurons displayed an increase in threshold and membrane resistance compared to uninjured neurons, while the resting membrane potential and action potential amplitude did not change significantly. Additionally, between 1-3 weeks post lesion, RS neurons displayed bursting or high spike frequency adaptation (SFA) firing patterns in response to applied depolarizing current. Over the course of 4-8 weeks post axotomy, RS neurons displayed a gradual restoration of normal membrane properties, although at 8 weeks, the properties often had not fully returned to normal.
In other studies from our laboratory, it was found that a rostral spinal cord transection is a much stronger stimulus for axonal regeneration than a caudal transection (McClellan et al., 2005). Specifically, following a rostral spinal cord transection at 10% BL, significantly more descending brain neurons regenerated their axons through the lesion than following more caudal transections at 30% or 50% BL. Furthermore, locomotor muscle activity began to recover just below a rostral spinal transection after 2-3 weeks, while recovery of muscle activity below a caudal spinal transection took much longer. Finally, as stated above, following rostral spinal cord transections, the axons of most RS neurons regenerate for relatively short distances and presumably make synapses just below the lesion (Davis and McClellan, 1994a,b). From these data, we hypothesize that the degree to which a neuron is stimulated to regenerate is dependent, in part, on synaptic connections it makes, above and/or below a spinal cord lesion (McClellan, 1998).

Based on the above information, we hypothesize that rostral spinal cord transections will alter the biophysical properties of axotomized RS neurons to a greater degree than more caudal transections. In the present study, we tested this hypothesis by determining the biophysical properties of RS neurons following rostral and caudal spinal cord transections and comparing these to the properties of uninjured RS neurons. Briefly, following right hemi-transections in the rostral spinal cord, the majority of RS neurons with injured descending axons displayed a number of properties typical of injury (see above). In contrast, following right hemi-transections in the caudal spinal cord, the majority of RS neurons with injured descending axons displayed properties typical of normal, uninjured RS neurons. Thus, the present study suggests that rostral spinal cord
transections alter the biophysical properties of axotomized RS neurons to a greater degree than more caudal transections, and this may be causally linked to the greater axonal regeneration that follows rostral lesions compared to caudal injuries.
METHODS

Animal Care

Larval sea lamprey (Petromyzon marinus) were maintained in 2.5–10.0 gallon aquaria at 23–25°C and typically were fed weekly (Hanson et al., 1974). The procedures in this study have been approved by the Animal Use and Care Committee at the University of Missouri (protocol reference No. 1506-1).

Spinal Cord Lesions

Hemi-transections. Prior to setting up in vitro preparations, larval sea lampreys (82-147 mm, n = 68 animals) were anesthetized in tricaine methanesulfonate (MS222, ~100 mg/200 ml; Sigma Chemical Co., St. Louis, MO). Animals were pinned dorsal side up, and a 5 mm incision was made to expose the spinal cord at 10%, 30%, or 50% body length (BL, normalized distance from the anterior tip of the head). It should be noted that these levels of the spinal cord where lesions were made are ~3.8-5.3 mm (10% BL), ~24-25 mm (30% BL), and ~44-45 mm (50% BL) from Müller cells in the brain (assuming a “typical” 100 mm larval lamprey). The meninges were deflected, and a hemi-transection was performed on the right side of the spinal cord using fine forceps and iridectomy scissors. The meninges were repositioned, the incision was closed, and the animals were returned to their home aquaria. After a recovery time of 2, 4, or 6 weeks, animals were again anesthetized, and the brains and spinal cords were removed for intracellular recordings from Müller cells (Fig. 1A1; see below). Hemi-transections were performed so that the properties of RS neurons with axotomized axons on one side of the brain could be compared to those of neurons with uninjured axons on the other side of the brain.
Because of some variability in the extent of hemi-transections, a few right RS neurons were not injured and a few left RS neurons were injured. These experiments test the hypothesis that rostral hemi-transections (10% BL) result in a much greater change in biophysical properties of axotomized RS neurons than caudal hemi-transections (50% BL) (see Introduction). With increasing recovery times, we predict that the properties of axotomized RS neurons will begin to return to normal.

Lateral lesions. The spinal cord was exposed at 10% BL (93-106 mm, n = 3 animals), as described above, and the lateral spinal cord tracts and dorsal columns were lesioned with fine forceps. After a recovery time of 2 weeks, the brains and spinal cords were removed for intracellular recordings (Fig. 1A2). This lesion left the ventromedial projecting descending axons of Müller cells intact (Rovainen et al., 1973) but interrupted many of the ascending axons that have direct or indirect inputs to descending brain neurons (Dubuc et al., 1993; Vinay and Grillner, 1992; Vinay et al., 1998). Thus, these experiments tested the hypothesis that the altered biophysical properties of Müller cells were due to axotomy and not removal of ascending synaptic inputs.

Spinal ablations. The spinal cord was exposed from 10-15% BL (94-115 mm, n = 10), as described above, a ~5 mm section of spinal cord was removed, and a small piece of Gelfoam (Upjohn, Kalamazoo, MI) was inserted in the gap between the ends of the spinal cord to prevent axonal regeneration and reconnection with spinal targets below the lesion. The incision was closed; the animals were allowed to recover for 8 weeks, at which time the brains and spinal cords were removed for electrophysiological recordings (Fig. 1A3). The purpose of these experiments was to test the hypothesis that prevention
of reconnection of regenerating descending axons with spinal targets prevents the restoration of normal biophysical properties of RS neurons.

Spinal transections. The spinal cord was exposed at 10% BL (93-108 mm, n = 6), as described above, and the spinal cord was completely transected with iridectomy scissors. After an 8-week recovery time and regeneration of descending axons, the brains and spinal cords were removed for intracellular recordings (Fig. 1A4). These experiments tested the hypothesis that regeneration of descending axons and reconnection with spinal targets is important for the restoration of normal biophysical properties.

Intracellular Recording

After the various recovery times described above, the brains and spinal cords were removed using a dissection procedure similar to that previously described (Davis and McClellan, 1994a). The isolated brains and spinal cords were pinned dorsal side up on a small rectangular strip of Sylgard (Corning Co.; Midland, MI) that was pinned to the bottom of a recording chamber containing cooled Ringer’s solution (McClellan, 1990). Intracellular micropipettes were pulled with a horizontal puller (Model P-80; Sutter Instruments, Novato, CA), filled with 5 M KAc (75-100 MΩ), and inserted into a microelectrode holder containing 3 M KCl. The electrode holder was inserted into the probe of an intracellular amplifier (Axoclamp 2A; Axon Instruments, Foster City, CA) that was mounted on a motorized manipulator (Newport, Inc.; Irvine, CA) having a minimum resolution of 0.05 µm. Intracellular recordings were made from uninjured and/or axotomized identified Müller cells in the MRN (M2, M3), ARRN (I1), and the MRRN (B1, B3, B4) (Fig. 1B). One suction electrode (SC1, see Fig. 1A1-1A4; tip diameter ~0.5 mm) was placed on the dorsal surface of the cord ~1 mm above the spinal
cord injury. A second suction electrode (SC2) was used to record from the caudal spinal cord ~1-2 mm below the injury (Fig. 1, A1-A4). These spinal cord electrodes were used to record orthodromic or elicit antidromic responses to test whether descending axons from axotomized RS neurons projected up to as well as beyond the injury site.

After impaling a neuron, hyperpolarizing current often was applied until stabilization of the resting membrane potential, which for the majority of recorded neurons was more negative than –70 mV, and no neurons were analyzed that had values less negative than –65 mV (see Table 1-5). In the “Bridge” mode of the intracellular amplifier, neurons were stimulated with 0.1-10 ms, +10 nA depolarizing pulses to elicit action potentials and to test for orthodromic responses in spinal cord electrodes (SC1 and SC2, see Fig. 1A1-1A4). In the discontinuous current clamp mode (“DCC”, $f_\text{s} \geq 4.2$ kHz min.), 200 ms depolarizing (+0.5 nA up to threshold current) and then hyperpolarizing (-0.5 to –3.0 nA) current pulses were applied and used for determining membrane resistance ($R_m$, see below) as well as the threshold for action potentials ($V_{th}$). Subsequently, the firing properties were determined by applying 2 s depolarizing current pulses that varied from the threshold current up to +10 nA.

**Biophysical Properties**

During the experiment, neurophysiological signals were recorded on tape (DR-890 Neurocorder; Cygnus Technologies Inc.; Delaware Water Gap, PA; 11kHz sampling rate per channel), and after the experiments, the data were acquired using a custom data acquisition and analysis system. In addition to the acquisition of intracellular voltage and current, spinal cord activity (SC1, SC2), and stimulus sync pulses, the instantaneous firing frequency of action potentials was generated by a custom electronic device and
stored on one of the acquisition channels (see “F” in Fig. 3A1; Rouse et al., 1998). The following neurophysiological parameters were measured (Fig. 2): (1) The resting membrane potential \(V_{\text{rest}}\) was defined as the baseline when the membrane potential was stable and constant. (2) The threshold voltage for action potentials \(V_{\text{th}}\) was the difference in voltage between \(V_{\text{rest}}\) and the value at which the voltage flattens before or after an action potential (Fig. 2B; DCC mode). (3) Action potential amplitude \(V_{\text{ap}}\) was defined as the difference in voltage between \(V_{\text{rest}}\) and the peak of the action potential (Fig 2A; “Bridge” mode). (4) The amplitudes of the fast (fAHP) and slow (sAHP) afterhyperpolarization were measured as the difference in voltage between \(V_{\text{rest}}\) and the most hyperpolarized point of these components of the AHP (Fig 2C; in the “Bridge” mode). Some neurons had an intermediate afterdepolarization (iADP) that occurred very quickly after the fAHP (Cangiano et al. 2002) (Fig 3Aiii, 4Biii, 6Aiii). Since long hyperpolarizing current pulses do not result in a “sag” in membrane potential, and short hyperpolarizing current pulses are not followed by depolarization, the iADP does not appear to be due to h-current (also see Cangiano et al. 2002). Preliminary modeling studies suggest that the iADP may be due to slow closing of calcium channels that remain partly open after the fAHP and before the sAHP develops (Kovalenko and McClellan, unpublished). Since the iADP probably prevented the fAHP from reaching its true amplitude, the fAHPS of those particular neurons were not measured or included in the statistical analysis. (5) Membrane resistance \(R_{\text{m}}\) was calculated as membrane
hyperpolarization divided by the smallest amount of applied hyperpolarizing current ($\Delta V_m/I_m$).

Criteria for Categorizing the Properties of Injured and Uninjured RS Neurons

First, under different conditions (i.e. lesion location, recovery time; see Fig. 1A1), RS neurons were first divided into those that had uninjured axons (i.e. orthodromic spike recorded below the spinal cord lesion; SC2, see Fig. 1A1) or injured axons (i.e. no spike recorded from SC2 electrode). It should be noted that because of some variability in the extent of hemi-transections, not all right RS neuron axons were injured and not all left RS neuron axons were uninjured. Second, during 2 s depolarizing current pulses (see Fig. 3A1, 3B1), firing patterns ($V$) and instantaneous firing frequency ($F$) of RS neurons were used to divide neurons into the following categories (firing frequency during the first and last 0.5 s of the 2 s current pulse were excluded): (1) Regular – firing frequency displayed a gradual decrease of no more than 40%, and only one instantaneous firing frequency was below 60% of the maximum (Fig. 3A1, 5B1, 6A1, 7A1; see “V” and “F”). (2) Irregular – two or more instantaneous firing frequencies were below 60% of the maximum frequency. (3) Bursting – some instantaneous firing frequencies were below 50% of the maximum frequency and were then followed by spike frequencies that were 75-100% of maximum (Fig. 4B1, 5C1, 7B1). (4) High spike frequency adaptation (SFA) – neurons fired several action potentials at the beginning of the 2 s depolarizing current pulse and then stopped firing during the remainder of the pulse (Fig. 3B1, 4A1, 5A1, 6B1). To be assigned to any one of the categories, neurons had to consistently display the same firing pattern for at least two depolarizing current pulses of different amplitudes.
Statistics

Parameters for the biophysical properties of RS neurons in the various uninjured and injured groups were compared either by standard one-way analysis of variance (ANOVA), with Tukey-Krammer multiple comparisons post-test, or the nonparametric Kruskal-Wallis Test, with Dunn’s Multiple Comparisons Test post-test. Statistical significance was determined by $p \leq 0.05$. 
Figure 1. Isolated brain/spinal cord preparation showing the brain (left) and rostral spinal cord, intracellular recording micropipette (IC), suction electrode (SC1) on the dorsal surface of the spinal cord rostral to the injury (e.g. HT), and suction electrode on the caudal spinal cord below the injury (SC2). (A1) Right spinal cord hemi-transection (HT) at 10%, 30%, or 50% body length (BL; normalized distance from the head). (A2) Lesions of the lateral tracts and dorsal columns at 10% BL disrupt a substantial portion of ascending axons. (A3) Ablation preparation with ~5 mm of spinal cord removed at 10-15% BL and Gelfoam inserted in the lesion site (white hatch marks). (A4) Complete transection (T) of the spinal cord at 10% BL. (B) Diagram of the brain showing contours around reticular nuclei: mesencephalic reticular nuclei (MRN), and anterior (ARRN), middle (MRRN), and posterior (PRRN) rhombencephalic reticular nuclei. Large identified Müller and Mauthner cells (dots): M1-M3 (MRN); I1-I4 (ARRN); B1-B5 (MRRN); and Mau and AM (MRRN).
A1 Right Hemi-Transections

A2 Lateral and Dorsal Column Lesions

A3 Spinal Cord Ablations

A4 Spinal Cord Transections

B Location of Müller Cells

Figure 1
Figure 2. Example of the biophysical properties of a representative large identified reticulospinal neurons (“II” Müller cell, see Fig. 1E). (A) Measurement of action potential amplitude ($V_{ap}$) and resting membrane potential ($V_{rest}$). (B) Measurement of spike threshold ($V_{th}$). (C) Measurement of the fast (fAHP) and slow (sAHP) afterhyperpolarization relative to $V_{rest}$. Vertical/ horizontal scale bar = (A) 40 mV/ 50 ms; (B) 76 mV/ 1.7s; and (C) 3.1 mV/ 100 ms.
Figure 2
RESULTS

Biophysical Properties of Uninjured and Axotomized RS Neurons

All Müller cells have ipsilateral axons (Rovainen, 1979). Therefore, a right hemi-transection (Fig. 1A1) should axotomize axons of all Müller cells on the right half of the brain, and all axons of Müller cells on the left side of the brain should be uninjured (however, see below). Depolarizing current pulses applied to Müller cells on the left side of the brain with uninjured axons (Fig. 3Aii) elicited a smooth, regular train of action potentials with very modest spike frequency adaptation (Fig. 3Ai). In addition, the afterhyperpolarization (AHP) of action potentials for these neurons displayed a fast AHP (fAHP) followed by a slow AHP (sAHP) (arrow and *, respectively, in Fig. 3Aiii). In some Müller cells, an intermediate afterdepolarization (iAHP) occurred between the fAHP and sAHP (Fig. 3Aiii). Finally, action potentials elicited in Müller cells with uninjured axons (see Methods) resulted in orthodromic spikes in the spinal cord above (SC1) and below (SC2) the injury site (Fig. 3Aii; see Fig. 1A1).

Previous studies have shown that following a hemi-spinal cord transection (HT) at 10% BL, the changes in biophysical properties of injured Müller cells are maximal at about 2 weeks post-injury (McClellan et al., 2002). In the present study, 2 weeks after a right hemi-transection at 10% BL, about 65% of Müller cells on the right side of the brain with axotomized spinal axons fired a single short burst (Fig. 3Bi, 4Ai; “high SFA”, see Methods) while about 35% fired very short, repetitive bursts (not shown, but similar to Fig. 4Ci; “bursting”, see Methods) during 2 s depolarizing current pulses (Fig. 9A). In addition, action potentials in neurons with axotomized descending axons tended to be
slightly longer than those for uninjured neurons, and orthodromic responses were not present below the lesion (Fig. 3Bii). Orthodromic responses often were present above the lesion but sometimes these were missing (Fig. 3Bii), presumably because of retraction of injured axons. Finally, action potentials of axotomized Müller cells had an augmented fAHP (Fig. 3Biii, arrow), as well as an sAHP that was absent or significantly reduced.

**Rationale for Location of Hemi-Transections**

Previous work has shown that rostral spinal transections are a stronger stimulus for axonal regeneration of RS neurons than caudal transections (McClellan, 2005). For example, 6 weeks after a spinal cord transection at 10% BL (50% BL), about 50% (0%) of injured axons of RS neurons have regenerated for ~10 mm below the lesion. We hypothesize that the degree to which an injured RS neuron is stimulated to regenerate depends, in part, on synapses it makes, either above or below a lesion (McClellan, 1998). It might be expected that the level of spinal cord injury would affect the degree to which axotomized Müller cells display the above altered biophysical properties. To test this notion, right hemi-transections were performed at rostral (10% BL), middle (30% BL), and caudal (50% BL) levels of the spinal cord.

At both 4 and 6 week recovery times, orthodromic spikes recorded in the spinal cord below the injury site (SC2, Fig. 1A1) could be due either to descending axons that were never injured or axons that had regenerated. Therefore, to avoid this uncertainty, control RS neurons were designated as those neurons that could elicit orthodromic responses below the lesion following a right hemi-transection at any of the three levels along the spinal cord and at 2 week recovery times, before substantial regeneration could have occurred. At this early recovery time, it can be assumed that the descending axons
of RS neurons satisfying these criteria were originally uninjured. The resting membrane potential (Vrest), input resistance (Rm), threshold (Vth), action potential amplitude (Vap), fAHP, and sAHP were measured for neurons with uninjured descending axons (Control) and injured descending axons (Injured) (Table 1). To maximize possible differences, only injured RS neurons for HT’s at 10% and 50% BL were considered (other data not used for this comparison are shown in Table 2).

**Effects of Axotomy at Different Levels of the Spinal Cord**

Early recovery times. Two weeks after a hemi-transection at 10% BL, the majority of RS neurons on the right side with injured descending axons displayed either a high SFA firing pattern (Fig 4Ai) or bursting (not shown). In contrast, 2 weeks after hemi-transections at 50% BL, almost 80% of RS neurons with injured descending axons displayed smooth, regular firing patterns (Fig 4Bi), while the remainder of those neurons displayed irregular, high SFA, or bursting firing patterns (Fig 4Ci, Fig. 9A). Most of these latter RS neurons that were axotomized at 50% BL had fAHPs and sAHPs that were similar to those of neurons with uninjured axons (Fig. 4Biii; “Injured: 50%, 2 weeks” vs “Control”, Table 1). In addition, for RS neurons whose axons were injured at 50% BL, the fAHP was smaller (p≤0.001) and the sAHP was larger (p≤0.001) than for neurons that were axotomized rostrally (Fig 4Ciii, Fig 4Aiii; “Injured: 50%, 2 weeks” vs “Injured: 10%, 2 weeks”, Table 1). Other biophysical properties were not significantly different at early recovery times.

It should be noted that in about half of the RS neurons with injured axons, the fAHP appeared to include both a fast component followed by a slower component that gradually returned the membrane potential to Vrest over the course of ~20-200 ms (Fig.
This slower component does not appear to be a modified sAHP, because in spinal neurons, blocking the sAHP with cadmium or apamin still results in a slow decaying hyperpolarization following the iADP (Cangiano et al., 2002). Thus, in the present study, the slow component of the fAHP may be due to a slow potassium channel that is different than those producing the main, fast component of the fAHP. Further experiments are needed to address this issue.

Long Recovery Times. Six weeks after a hemi-transection at 10% BL, the majority of RS neurons with injured descending axons displayed a high SFA firing pattern (Fig 5Ai) while the remaining neurons showed regular (Fig 5Bi), irregular, or bursting firing patterns (Fig 7C). Conversely, 81% of neurons with descending axons that were injured at 50% BL displayed regular firing patterns (Fig 6Ai) while the remaining neurons showed bursting (Fig 6Bi) and high SFA firing patterns (Fig 9C). RS neurons with descending axons injured at 10% BL (Fig 5 A, Biii) had a larger fAHP \( (p \leq 0.001) \) and a smaller sAHP \( (p \leq 0.001) \) than for neurons that were injured at 50% BL (Fig 6A, Biii; “Injured: 10%, 6 weeks” vs “Injured: 50%, 6 weeks”, Table 1). Other biophysical properties were not significantly different at these long recovery times.

Effects of Increasing Recovery Times for a Given Location of Axotomy

After axotomy at a given level of the spinal cord (10%, 30%, or 50% BL), we compared the firing patterns of RS neurons at increasing recovery times (2, 4, and 6 weeks). Neurons were categorized based on (1) uninjured or injured descending axon, as indicated by the presence or absence, respectively, of orthromic responses below the lesion (SC2, Fig. 1A1), (2) location in the brain (left or right side), and (3) the firing pattern (regular, irregular, bursting, or high SFA) (see Methods).
Hemi-transections at 10% BL. Although left RS neurons with uninjured descending axons displayed some differences in firing patterns for right hemi-transections at different levels, almost all of these neurons displayed regular firing patterns (n = 51/57). Because left RS neurons with injured descending axons displayed very similar firing patterns as right RS neurons with injured descending axons, the numbers of neurons were pooled together. Almost all of the RS neurons on the right side of the brain had injured descending axons. First, at 2 week recovery times, the majority of neurons (right or left) with injured descending axons displayed high SFA (65%), while most of the remaining 35% of neurons displayed bursting (Fig 7A). Second, at 4 weeks, the majority of neurons with injured descending axons displayed bursting (52%), 24% exhibited high SFA, 14% displayed regular firing, and 10% fired irregular patterns of action potentials (Fig 7B). Third, at 6 weeks, the majority of neurons with injured descending axons displayed high SFA (54%), with 23% displaying bursting, 14% displaying regular firing, and 9% displaying irregular firing patterns (Fig 7C). Thus, with increasing recovery times following hemi-transections at 10% BL, some RS neurons with injured descending axons began to display regular or irregular firing patterns.

Hemi-transections at 30% BL. Virtually all RS neurons with uninjured descending axons displayed regular firing patterns (n = 42/45). Almost all right RS descending axons were injured (n = 81/84), as indicated by the lack of orthodromic responses caudal to the lesion (SC2; Fig. 1A1). At all recovery times, a substantial fraction of neurons (left or right) with injured descending axons displayed a regular firing patterns: 61% at 2 weeks (Fig 8A); 54% at 4 weeks (Fig 8B); and 50% at 6 weeks (Fig 8C). Neurons with injured descending axons displaying irregular firing pattern were only
observed at 2 week recovery times (Fig 8A). With increasing recovery times, greater numbers of RS neurons with injured descending axons displayed a high SFA firing pattern: 9% at 2 weeks (Fig 8A); 17% at 4 weeks (Fig 8B); and 33% at 6 weeks (Fig 8C). Thus, with increasing recovery times (2Æ6 wks) following a right hemi-transection at 30% BL, the percentages RS neurons with injured descending axons displaying regular firing patterns decreased, while the percentages of these RS neurons displaying high SFA firing patterns increased by a factor of four.

Hemi-transections at 50% BL. A very high percentage of RS neurons with uninjured descending axons showed regular firing patterns (n = 48/53). At all recovery times, the majority of RS neurons with injured descending axons displayed regular firing patterns: 78% at 2 weeks (Fig 9A), 55% at 4 weeks (Fig 9B), and 81% at 6 weeks (Fig 9C). The percentages of neurons with injured descending axons that displayed bursting or high SFA firing patterns increased from 2 weeks (9% or 6%, respectively) to 4 weeks (30% or 10%), and then decreased at 6 weeks (16% or 3%).

Summary of effects of increasing recovery times. At all recovery times (2, 4, and 6 weeks), the fAHPs of RS neurons with descending axons that were injured at 10% BL were significantly larger compared to those in uninjured, control neurons (p≤0.01) and those in neurons that were axotomized at 50% (p≤0.01). The sAHPs were significantly smaller in neurons with descending axons that were injured at 10% BL compared to those in control neurons (p≤0.001). At 2 and 6 weeks, the sAHPs of neurons with descending axons that were injured at 50% BL were significantly larger than those at the same recovery times following injury at 10% BL (p≤0.001). Additionally, the sAHPs of neurons with descending axons injured at 50% that had recovered for 4 weeks were
significantly smaller than those that had recovered for 6 weeks (p ≤ 0.05), but not 2 weeks (Table 1).

**Correlations of Biophysical Properties with Firing Pattern**

After examining the histograms for firing patterns (Fig 7-9), it was apparent that the firing patterns were not necessarily dependant on whether an orthodromic response could be recorded below the lesion. First, a neuron whose descending axon was injured at 50% BL and did not regenerate might have had a regular firing pattern because the neuron made additional synapses above the lesion. Second, a neuron that elicited orthodromic responses below a lesion but had a high SFA or bursting firing pattern might have been transiently or partially injured, but the axon may have then sealed. Alternatively, the descending axon may have been injured and then regenerated a short distance below the lesion, but not far enough to make sufficient numbers of synapses to restore normal biophysical properties. Third, although only the AHPs were significantly different between experimental groups (Table 1), there appeared to be a correlation between the enhanced fAHP/decreased sAHP and the altered firing patterns of neurons (high SFA or bursting).

To examine if there was a relationship between the firing patterns and the AHPs, additional analysis was performed that ignored whether an orthodromic response could be elicited below the lesion, which in the above analysis was used as an indicator of injury (Table 3). Neurons were grouped according to the level of axotomy (10% or 50%; 30% was not included to maximize possible differences), recovery time, and whether they displayed a regular firing pattern or bursting/high SFA. Neurons that displayed bursting or high SFA were grouped together because they represent conditions of
maximal injury (Fig 7A). This analysis indicated that there were no significant
differences between the resting membrane potential, input resistance, threshold, and
action potential amplitude of the various groups (not included in Table 3). The main
differences were for the fAHP and sAHP, and these are summarized in Table 3. (1)
Following HTs at 10% BL and a 2 week recovery time, neurons that displayed bursting
or high SFA firing patterns had fAHPs that were significantly larger and sAHPs that were
significantly smaller than those from neurons with regular firing patterns. In addition,
following HTs at 10% BL and 4 week recovery times, sAHPs were significantly smaller
than those for neurons with regular firing patterns. (2) For neurons with regular firing
patterns, regardless of differences in axotomy location or recovery time, there were no
significant difference between the fAHPs and sAHPs. (3) For neurons from animals that
had a HT at 10% BL and displayed bursting or high SFA firing patterns, the AHPs were
not significantly different at the different recovery times. (4) Similarly, in animals with
HTs at 50% BL, AHPs were not significantly different in neurons that displayed high
SFA/bursting. (5) At a given recovery time, the AHPs of neurons that displayed bursting
or high SFA firing pattern following HTs at 10% were not significantly different than
those from neurons following HTs at 50% BL.

Lateral Lesions

Most of the descending axons of Müller cells project in the ventromedial spinal
cord tracts (Rovainen, 1979). Therefore, lateral lesions and dorsal column lesions at 10%
BL were made (Fig 1A2) to spare the axons of these RS neurons but lesion many
ascending axons that have direct or indirect inputs to descending brain neurons (Dubuc et
al., 1993; Vinay and Grillner, 1992; Vinay et al., 1998). The purpose of these
experiments was to test whether the altered biophysical properties of injured Müller cells were due to axotomy or removal of ascending synaptic inputs. Following lateral and dorsal column spinal cord lesions, most of the RS neurons that had an uninjured descending axon (13 of 19 neurons), as indicated by orthodromic responses above and below the lesion (Fig. 10Aii), displayed regular firing patterns (n = 7/8; Fig 10Ai) and AHPs with two distinct components (fAHP, arrow; sAHP, *; Fig. 10Aiii) similar to neurons with uninjured descending axons following right hemi-transections (Fig 3Aiii).

Following lateral and dorsal column spinal cord lesions (Fig. 1A2), RS neurons with injured descending axons (6 of 19), as indicated by the absence of orthodromic responses below the lesion (SC2), displayed either high SFA firing patterns (n = 4, Fig 10Bi) or bursting (n = 2). In about half of these injured RS neurons, orthodromic spikes could be evoked above the lesion site (SC1, Fig. 10Bii). These RS neurons had an augmented fAHP and sAHPs that were absent or significantly reduced (p ≤ 0.05) (Fig 10Biii, Table 4), similar to neurons displaying injury-type firing patterns following HTs (Fig 3Biii). In fact, the fAHP and sAHP of neurons with a given firing pattern were not significantly different in animals with lateral lesions or those with right hemi-transections.

It should be noted that because of some variability in the extent of hemi-transections, not all RS neurons on the right side of the brain had injured axons and not all RS neurons on the left side had uninjured axons. When RS neurons on the left side of the brain were injured, they displayed properties similar to the RS neurons on the right side of the brain that were injured. In addition, left RS neurons that were injured displayed properties similar to injured neurons from animals with lateral and dorsal
column lesions. Thus, these results strongly suggest that the changes in biophysical properties and firing patterns were due to axotomy of descending axons and not general effects, such as interruption of ascending inputs or diffusible substances.

**Spinal Cord Ablation vs. Transection**

The purpose of the ablation experiments was to prevent regeneration and reconnection of descending axons with spinal targets below the lesion (see Methods). We hypothesize that this manipulation should prevent the restoration of normal biophysical properties and firing patterns of RS neurons. No obvious axonal regeneration was observed through the ablation site, which contained Gelfoam (see Methods), and this was confirmed in all RS neurons by the absence of orthodromic response below the injury site (SC2, Fig 1A3). Under these conditions, in approximately half of the RS neurons (10/19), orthodromic spikes could be evoked above the injury site (SC1). Most of the RS neurons displayed a high SFA firing pattern (42%), while the remaining neurons displayed bursting (32%), and regular (26%) firing patterns (Fig 11A).

Following spinal cord transections at 10% BL, injured descending axons could regenerate and potentially reconnect with spinal targets below the lesion, allowing us to test whether these factors were important for the restoration of normal properties. Stimulation of the majority of RS neurons (10/14) evoked orthodromic responses above (SC1) and below (SC2) the transection site (Fig 1A4, “Regenerated”, Fig 11B), indicating that some of these injured descending axons had regenerated. The majority of RS neurons with regenerated axons displayed bursting firing patterns (60%), while the remainder displayed regular (10%) and high SFA (30%) firing patterns (Fig 11B). Of the RS neurons that did not regenerate their axons below the lesion, half displayed regular
firing patterns and 25% displayed bursting or high SFA. In most of these neurons (3/4), it was possible to evoke an orthodromic spike above the transection site (SC1, Fig 1A4).

For animals with spinal ablations or full spinal transections at 10% BL, the condition of RS neurons was very poor. Most RS neurons in the brain were swollen and literally popped out from the brain tissue. Some neurons could be impaled but had no recordable resting membrane potential. The 10 lamprey with spinal cord ablation injuries should have provided a potential of 120 neurons (12 Müller cells in each brain; see Fig. 1B) from which to record, but only 19 were sufficiently healthy to impale for intracellular recordings. Of the 6 lamprey with spinal transections at 10% BL, only 14 RS neurons of 72 possible cells could be recorded.

For statistical comparisons, RS neurons were separated according to lesion type (ablation or transection) and firing pattern, since the level of the lesions and recovery times were identical for both types of injuries (Table 5). There were no significant differences between the resting membrane potential, input resistance, threshold, action potential amplitude, or fAHP amplitude of RS neurons in the various experimental groups. For RS neurons that had regular firing patterns following full transections at 10% BL, there were too few cells with negative fAHPs (see Methods) to statistically compare to those of neurons in other categories. However, the sAHPs of these neurons were significantly larger than those for neurons that displayed regular firing patterns after an ablation injury (p<0.001) and those for neurons that displayed bursting or high SFA after spinal transections (p<0.001). For RS neurons that had bursting or high SFA firing patterns, regardless of the injury type, there were no significant differences between the sAHPs.
Figure 3. (A) Uninjured and (B) axotomized RS neurons 2 weeks after a hemi-transection at 10% BL. (Ai, Bi) Membrane potential (V), current (I), and instantaneous firing frequency (F) in response to depolarizing current pulses. (Aii, Bii) Orthodromically-elicited action potentials (top traces) and recordings from the spinal cord electrodes (see Fig. 1A1) showing responses above (SC1) and below (SC2) the lesion for neuron in Aii (i.e. uninjured) but no spinal cord responses for neuron in Bii (injured) (see Results). (Aiii, Biii) Fast (fAHP, arrow) and slow (sAHP, asterisk) afterhyperpolarization components of the action potentials shown in Aii and Bii. (Aiii) In some neurons, there was an intermediate afterdepolarization (iADP) between the fAHP and sAHP. Vertical/horizontal scale bar = (Ai, Bi) 100mV, 7.8 nA, 55.5 Hz/ 875 ms; (Aii, Bii) 51 mV/ 2 ms; (Aiii, Biii) 5.1 mV/ 47 ms.
Figure 3
Figure 4. Axotomized right “B1” Müller cells 2 weeks after a hemi-transection at (A) 10% BL or (B, C) 50% BL. (Ai, Bi, Ci) Membrane potential (V), current (I), and instantaneous firing frequency (F) in response to depolarizing current pulses. (Aii, Bii, Cii) Action potentials (top traces) and recordings from the spinal cord (see Fig. 1A1) showing the absence of orthodromic responses above (SC1) and below (SC2) the lesion site. (Aiii, Biii, Ciii) Fast (fAHP, arrows) and slow afterhyperpolarization (sAHP, asterisk) of the action potentials shown in Aii, Bii, and Cii. (Biii) In some neurons, there was an intermediate afterdepolarization (iADP) between the fAHP and sAHP. Vertical/Horizontal scale bar = (Ai, Bi, Ci) 100 mV, 6.8 nA, 29 Hz/870 ms; (Aii, Bii, Cii) 44 mV/2 ms; (Aiii, Biii, Ciii) 3.5 mV/47 ms.
Figure 4
Figure 5. Two examples of axotomized right “M3” Müller cells 6 weeks after a hemi-transection at 10% BL. (Ai, Bi) Membrane potential (V), current (I), and instantaneous firing frequency (F) in response to depolarizing current pulses. (Aii, Bii) Action potentials (top traces) and absence of responses in the spinal cord (SC1 and SC2), indicating injured descending axons. (Aiii, Biii) The fast afterhyperpolarization (fAHP) of action potentials in Aii, Bii showing the absence of the sAHP. Vertical/ Horizontal scale bar = (Ai, Bi) 100mV, 7.3 nA, 25 Hz/ 880 ms; (Aii, Bii) 53 mV/ 2 ms; (Aiii, Biii) 3.2 mV/ 47 ms.
Figure 5
Figure 6. Two examples of axotomized right “M3” Müller cells 6 weeks after a hemi-transection at 50% BL. (Ai, Bi) Membrane potential (V), current (I), and instantaneous firing frequency (F) in response to depolarizing current pulses. (Aii, Bii) Action potentials (upper traces) and recordings from the spinal cord (SC1 and SC2; see Fig. 3). (Aiii, Biii) Fast afterhyperpolarization (fAHP, arrow) and slow afterhyperpolarization (sAHP, asterisk) of action potentials from Aii and Bii. (Aiii) One of the neurons had an intermediate afterdepolarization (iADP). Vertical/ Horizontal scale bar = (Ai, Bi) 100 mV, 4.7 nA, 15.5 Hz/ 880 ms; (Aii, Bii) 45 mV/ 2 ms; (Aiii, Biii) 2.85 mV/ 47 ms.
**Figure 7.** Uninjured and injured RS neurons on the left and right sides of the brain showing the proportion of cells with different firing properties from animals with right hemi-transections at 10% BL and recovery times of (A) 2 weeks, (B) 4 weeks, and (C) 6 weeks.
Figure 7
**Figure 8.** Uninjured and injured RS neurons on the left and right sides of the brain showing the proportion of cells with different firing properties from animals with right hemi-transections at 30% BL and recovery times of (A) 2 weeks, (B) 4 weeks, and (C) 6 weeks.
Figure 8
Figure 9. Uninjured and injured RS neurons on the left and right sides of the brain showing the proportion of cells with different firing properties from animals with right hemi-transections at 50% BL and recovery times of (A) 2 weeks, (B) 4 weeks, and (C) 6 weeks.
Figure 9
Figure 10. (A) Uninjured and (B) axotomized “B3” Müller cells 2 weeks after a lesion of the lateral tracts and dorsal columns at 10% BL. (Ai, Bi) Membrane potential (V), current (I), and instantaneous firing frequency (F) in response to depolarizing current pulses. (Aii, Bii) Orthodromically-elicited action potentials (top traces) and recordings from the spinal cord electrodes (see Fig. 1A2) showing responses above (SC1) and below (SC2) the lesion for neuron in Aii (i.e. uninjured) but only a response above the lesion for neuron in Bii (injured) (see Results). (Aiii, Biii) Fast (fAHP, arrow) and slow (sAHP, asterisk) afterhyperpolarization components of the action potentials shown in Aii and Bii. Vertical/horizontal scale bar = (Ai, Bi) 100mV, 7.5 nA, 67 Hz/ 875 ms; (Aii, Bii) 44.2 mV/ 2 ms; (Aiii, Biii) 4.4 mV/ 47 ms.
Figure 10
**Figure 11.** Uninjured and injured RS neurons on the left and right sides of the brain showing the proportion of cells with different firing properties from animals with (A) ablations and (B) transections at 10% BL, both with 8 week recovery times.
Figure 11

A

Ablation

Number of RS Neurons

![Graph showing the number of RS Neurons for Ablation with categories: Left Regenerated Axon, Right Regenerated Axon, Left Non-regenerated Axon, Right Non-regenerated Axon.]

B

10% BL Transection

Number of RS Neurons

![Graph showing the number of RS Neurons for 10% BL Transection with categories: Left Regenerated Axon, Right Regenerated Axon, Left Non-regenerated Axon, Right Non-regenerated Axon.]

Legend:
- Black: High SFA
- Gray: Bursting
- Dashed: Irregular
- White: Regular
TABLE 1
Effects of Rostral Axotomy (10% BL) vs. Caudal Axotomy (50%) on Biophysical Properties of RS Neurons
at Different Recovery Times

<table>
<thead>
<tr>
<th></th>
<th>Vrest (mV)</th>
<th>Rm (MΩ)</th>
<th>Vth (mV)</th>
<th>Vap (mV)</th>
<th>fAHP (mV)</th>
<th>sAHP (mV)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Uninjured, 2 weeks)</td>
<td>-75.5 ± 4.72a</td>
<td>7.25 ± 3.07</td>
<td>16.4 ± 6.62</td>
<td>103.7 ± 6.59</td>
<td>4.15 ± 3.28</td>
<td>1.30 ± 1.16</td>
<td>50</td>
</tr>
<tr>
<td>Injured: 10%, 2 weeks</td>
<td>-74.2 ± 5.21</td>
<td>8.00 ± 4.11</td>
<td>16.5 ± 7.38</td>
<td>105.9 ± 9.56</td>
<td>11.9 ± 4.63***</td>
<td>0.16 ± 1.06 ***</td>
<td>57</td>
</tr>
<tr>
<td>Injured: 10%, 4 weeks</td>
<td>-74.6 ± 5.15</td>
<td>8.26 ± 3.40</td>
<td>21.5 ± 8.66</td>
<td>106.0 ± 12.3</td>
<td>9.36 ± 4.74**</td>
<td>0.15 ± 0.49 ***</td>
<td>21</td>
</tr>
<tr>
<td>Injured: 10%, 6 weeks</td>
<td>-73.4 ± 4.91</td>
<td>8.93 ± 5.61</td>
<td>17.8 ± 6.99</td>
<td>105.3 ± 8.85</td>
<td>9.55 ± 5.03***</td>
<td>0.19 ± 0.51 ***</td>
<td>35</td>
</tr>
<tr>
<td>Injured: 50%, 2 weeks</td>
<td>-76.4 ± 5.02</td>
<td>6.84 ± 2.75</td>
<td>18.5 ± 6.11</td>
<td>100.8 ± 11.7</td>
<td>3.73 ± 4.16†††</td>
<td>1.19 ± 1.16†††</td>
<td>32</td>
</tr>
<tr>
<td>Injured: 50%, 4 weeks</td>
<td>-73.6 ± 3.40</td>
<td>6.83 ± 3.47</td>
<td>16.9 ± 5.31</td>
<td>102.0 ± 9.89</td>
<td>3.60 ± 2.73††</td>
<td>0.77 ± 0.85 ‡</td>
<td>20</td>
</tr>
<tr>
<td>Injured: 50%, 6 weeks</td>
<td>-75.9 ± 4.82</td>
<td>8.56 ± 3.22</td>
<td>17.5 ± 5.52</td>
<td>103.5 ± 7.03</td>
<td>3.20 ± 2.28†††</td>
<td>1.87 ± 1.33†††</td>
<td>37</td>
</tr>
</tbody>
</table>

a - Values are means ± S.D.
* - p<0.05, ** - p<0.01, *** - p<0.001. Significantly different than control group (ANOVA).
†† - p<0.01, ††† - p<0.001. Significantly different compared to the same recovery time for hemi-transections at 10% BL (ANOVA).
‡ - p<0.05. Significantly different compared to 6 weeks, but not 2 weeks, for hemi-transections at 50% BL (ANOVA).
<table>
<thead>
<tr>
<th>Condition</th>
<th>Vrest (mV)</th>
<th>Rm (MΩ)</th>
<th>Vth (mV)</th>
<th>Vap (mV)</th>
<th>fAHP (mV)</th>
<th>sAHP (mV)</th>
<th>n = cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injured: 30%, 2 weeks</td>
<td>-74.1 ± 4.50^a</td>
<td>6.35 ± 2.92</td>
<td>16.6 ± 6.81</td>
<td>101.8 ± 12.0</td>
<td>4.97 ± 3.45</td>
<td>1.24 ± 1.39</td>
<td>67</td>
</tr>
<tr>
<td>Injured: 30%, 4 weeks</td>
<td>-74.3 ± 4.10</td>
<td>7.24 ± 2.36</td>
<td>19.8 ± 8.29</td>
<td>107.4 ± 7.79</td>
<td>5.38 ± 3.48</td>
<td>0.77 ± 0.91</td>
<td>24</td>
</tr>
<tr>
<td>Injured: 30%, 6 weeks</td>
<td>-75.0 ± 4.40</td>
<td>7.33 ± 2.63</td>
<td>20.9 ± 5.02</td>
<td>105.2 ± 10.1</td>
<td>4.80 ± 2.72</td>
<td>0.65 ± 1.06</td>
<td>18</td>
</tr>
<tr>
<td>Uninjured: 10%, 4 weeks</td>
<td>-75.1 ± 4.96</td>
<td>6.87 ± 3.19</td>
<td>16.6 ± 7.68</td>
<td>103.0 ± 11.6</td>
<td>4.17 ± 1.94</td>
<td>1.13 ± 1.15</td>
<td>24</td>
</tr>
<tr>
<td>Uninjured: 30%, 4 weeks</td>
<td>-72.7 ± 3.61</td>
<td>6.88 ± 2.26</td>
<td>18.9 ± 7.07</td>
<td>104.5 ± 7.67</td>
<td>1.90 ± 1.71</td>
<td>1.35 ± 0.71</td>
<td>20</td>
</tr>
<tr>
<td>Uninjured: 50%, 4 weeks</td>
<td>-74.8 ± 5.08</td>
<td>6.19 ± 2.43</td>
<td>15.9 ± 6.41</td>
<td>105.1 ± 5.98</td>
<td>3.48 ± 2.23</td>
<td>0.86 ± 1.01</td>
<td>19</td>
</tr>
<tr>
<td>Uninjured: 10%, 6 weeks</td>
<td>-74.8 ± 4.44</td>
<td>6.64 ± 3.17</td>
<td>18.7 ± 8.31</td>
<td>99.3 ± 8.98</td>
<td>4.63 ± 3.85</td>
<td>0.93 ± 0.85</td>
<td>12</td>
</tr>
<tr>
<td>Uninjured: 30%, 6 weeks</td>
<td>-73.2 ± 3.92</td>
<td>9.47 ± 5.19</td>
<td>17.4 ± 6.35</td>
<td>101.6 ± 8.89</td>
<td>4.70 ± 2.59</td>
<td>1.98 ± 1.32</td>
<td>9</td>
</tr>
<tr>
<td>Uninjured: 50%, 6 weeks</td>
<td>-75.1 ± 5.94</td>
<td>8.31 ± 2.98</td>
<td>16.4 ± 10.4</td>
<td>104.0 ± 11.9</td>
<td>3.89 ± 3.17</td>
<td>1.69 ± 1.18</td>
<td>23</td>
</tr>
</tbody>
</table>

^a Values are means ± S.D.
TABLE 3

Relationship Between Firing Pattern and Fast (fAHP) and Slow (sAHP) Afterhyperpolarizations of RS Neurons Following Axotomy at Different Levels of the Spinal Cord and at Different Recovery Times

<table>
<thead>
<tr>
<th>Firing Pattern</th>
<th>fAHP (mV)</th>
<th>sAHP (mV)</th>
<th>n = cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%, 2 weeks, Regular</td>
<td>4.24 ± 3.16^a</td>
<td>0.95 ± 1.15</td>
<td>22</td>
</tr>
<tr>
<td>10%, 2 weeks, Bursting + high SFA</td>
<td>12.0 ± 4.62 ***</td>
<td>0.17 ± 1.07*</td>
<td>56</td>
</tr>
<tr>
<td>10%, 4 weeks, Regular</td>
<td>5.50 ± 4.01</td>
<td>1.18 ± 1.16</td>
<td>24</td>
</tr>
<tr>
<td>10%, 4 weeks, Bursting + high SFA</td>
<td>8.48 ± 4.63</td>
<td>0.06 ± 0.26*</td>
<td>18</td>
</tr>
<tr>
<td>10%, 6 weeks, Regular</td>
<td>5.93 ± 3.65</td>
<td>0.88 ± 0.89</td>
<td>15</td>
</tr>
<tr>
<td>10%, 6 weeks, Bursting + high SFA</td>
<td>10.2 ± 5.22</td>
<td>0.10 ± 0.36</td>
<td>29</td>
</tr>
<tr>
<td>50%, 2 weeks, Regular</td>
<td>3.05 ± 3.11</td>
<td>1.40 ± 1.11</td>
<td>36</td>
</tr>
<tr>
<td>50%, 2 weeks, Bursting + high SFA</td>
<td>8.20 ± 7.57</td>
<td>0.00 ± 0.00</td>
<td>5</td>
</tr>
<tr>
<td>50%, 4 weeks, Regular</td>
<td>3.46 ± 1.98</td>
<td>0.99 ± 0.97</td>
<td>28</td>
</tr>
<tr>
<td>50%, 4 weeks, Bursting + high SFA</td>
<td>3.43 ± 3.52</td>
<td>0.29 ± 0.59</td>
<td>10</td>
</tr>
<tr>
<td>50%, 6 weeks, Regular</td>
<td>3.81 ± 2.61</td>
<td>1.95 ± 1.30</td>
<td>49</td>
</tr>
<tr>
<td>50%, 6 weeks, Bursting + high SFA</td>
<td>2.93 ± 3.53</td>
<td>1.03 ± 0.83</td>
<td>8</td>
</tr>
</tbody>
</table>

^- Values are means ± S.D.
^- * - p<0.05, *** - p<0.001. Significantly different compared to neurons displaying regular firing patterns at the same recovery time and % BL for hemi-transections (ANOVA).
### TABLE 4

**Biophysical Properties of RS Neurons Two Weeks After Lesions of the Lateral/Dorsal Spinal Cord Tracts at 10% BL**

**Compared to those Following Right Hemi-Transections**

<table>
<thead>
<tr>
<th></th>
<th>Vrest (mV)</th>
<th>Rm (MΩ)</th>
<th>Vth (mV)</th>
<th>Vap (mV)</th>
<th>fAHP (mV)</th>
<th>sAHP (mV)</th>
<th>n = cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral Lesion: 10%, 2 weeks, Regular</td>
<td>-76.59 ± 3.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.32 ± 1.87</td>
<td>14.09 ± 3.54</td>
<td>108.8 ± 5.46</td>
<td>3.89 ± 2.32</td>
<td>0.91 ± 0.35</td>
<td>12</td>
</tr>
<tr>
<td>Lateral Lesion: 10%, 2 weeks, Bursting + high SFA</td>
<td>-72.14 ± 5.73</td>
<td>5.71 ± 1.11</td>
<td>19.73 ± 5.70</td>
<td>105.4 ± 9.93</td>
<td>10.9 ± 5.39&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.26 ± 0.68&lt;sup&gt;**&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>Right Hemi-transection: 10%, 2 weeks, Regular</td>
<td>-75.96 ± 4.43</td>
<td>8.06 ± 3.74</td>
<td>14.4 ± 5.50</td>
<td>103.2 ± 7.39</td>
<td>4.24 ± 3.16</td>
<td>0.95 ± 1.15</td>
<td>22</td>
</tr>
<tr>
<td>Right Hemi-transection: 10%, 2 weeks, Bursting + high SFA</td>
<td>-74.18 ± 5.25</td>
<td>7.99 ± 4.15</td>
<td>16.49 ± 7.44</td>
<td>105.8 ± 9.62</td>
<td>12.0 ± 4.62&lt;sup&gt;†††&lt;/sup&gt;</td>
<td>0.17 ± 1.07&lt;sup&gt;†††&lt;/sup&gt;</td>
<td>56</td>
</tr>
</tbody>
</table>

<sup>a</sup> - Values are means ± S.D.

* - p<0.05, ** - p<0.01. Significantly different compared to neurons displaying regular firing patterns following lateral lesions (ANOVA).

<sup>†††</sup> - p<0.001. Significantly different compared to neurons displaying regular firing patterns following hemi-transections (ANOVA).
<table>
<thead>
<tr>
<th></th>
<th>Vrest (mV)</th>
<th>Rm (MΩ)</th>
<th>Vth (mV)</th>
<th>Vap (mV)</th>
<th>fAHP (mV)</th>
<th>sAHP (mV)</th>
<th>n = cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ablation: 10%, 8 weeks,</td>
<td>-78.3 ± 4.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.6 ± 10.5</td>
<td>19.9 ± 2.86</td>
<td>116.4 ± 5.13</td>
<td>4.52 ± 2.55</td>
<td>0.00 ± 0.00</td>
<td>5</td>
</tr>
<tr>
<td>Regular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ablation: 10%, 8 weeks,</td>
<td>-75.0 ± 7.40</td>
<td>9.91 ± 3.04</td>
<td>19.4 ± 9.19</td>
<td>109.8 ± 8.16</td>
<td>8.20 ± 4.81</td>
<td>0.05 ± 0.19</td>
<td>14</td>
</tr>
<tr>
<td>Bursting + high SFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transection: 10%, 8</td>
<td>-75.8 ± 5.42</td>
<td>10.2 ± 4.15</td>
<td>22.2 ± 7.29</td>
<td>110.3 ± 5.03</td>
<td>N/A</td>
<td>1.47 ± 0.64&lt;sup&gt;†††&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>weeks, Regular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transection: 10%, 8</td>
<td>-73.3 ± 4.94</td>
<td>7.95 ± 3.44</td>
<td>20.3 ± 5.18</td>
<td>104.0 ± 8.38</td>
<td>6.27 ± 4.35</td>
<td>0.14 ± 0.45&lt;sup&gt;***&lt;/sup&gt;</td>
<td>11</td>
</tr>
<tr>
<td>weeks, Bursting +</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>high SFA</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> - Values are means ± S.D.

<sup>***</sup> - p<0.001. Significantly different compared to neurons displaying regular firing patterns following transections (ANOVA).

<sup>†††</sup> - p<0.001. Significantly different compared to neurons displaying regular firing patterns following ablations (ANOVA).
DISCUSSION

Effects of Axotomy of Reticulospinal Neurons in the Lamprey

Following a right hemi-spinal cord transection at 10% BL and 2 week recovery times, the majority of RS neurons (65%) with injured descending axons displayed a high SFA firing pattern. In contrast, following a right hemi-transection at 50% BL and 6 week recovery times, the majority of RS neurons with injured descending axons (81%) displayed a regular firing pattern. At a given recovery time, RS neurons that were axotomized at 10% BL had significantly larger fAHPs and significantly smaller sAHPs compared to RS neurons at the same recovery times that were axotomized at 50% BL or compared to uninjured RS neurons. Furthermore, following right hemi-transections at 10% BL and 2 week recovery times, RS neurons that displayed bursting/high SFA firing patterns had significantly larger fAHPs and significantly smaller sAHPs than those that displayed regular firing patterns. Under similar conditions, but at 4 week recovery times, RS neurons that displayed bursting/high SFA had significantly smaller sAHPs than those that fired regular firing patterns. Six weeks after hemi-transections at 10% BL, some RS neurons with injured descending axons began to display regular or irregular firing patterns. In contrast, six weeks after hemi-transections at 30% BL, the percentages of RS neurons with injured descending axons displaying regular firing patterns decreased, while the percentages of RS neurons with injured descending axons displaying high SFA firing patterns increased by a factor of four. Finally, following right hemi-transections at 50% BL, the percentages of neurons with injured descending axons that displayed bursting or high SFA firing patterns increased from 2 weeks (9% and 6%, respectively) to 4 weeks
(30% and 10%), and then decreased by 6 weeks (16% and 3%). Perhaps with longer recovery times following lesions at 10% BL, the time course of recovery from injury might have become more clear, and a shift in time of maximal injury effects might have been seen with axotomies at more caudal locations.

Following lesions of the lateral spinal tracts and dorsal columns at 10% BL and 2 week recovery times, RS neurons that displayed bursting/high SFA firing patterns had significantly larger fAHP and significantly smaller sAHP than those that fired regular firing patterns, similar to the situation following right hemi-transections, as described above.

Following full spinal cord transections at 10% BL and 8 week recovery times, RS neurons that displayed regular firing patterns had sAHPs that were significantly larger than those that displayed bursting/ high SFA firing patterns. Also, the above RS neurons that displayed regular firing patterns had sAHPs that were significantly larger than those for RS neurons that fired regular firing patterns following spinal cord ablation injuries at 10% BL and 8 weeks recovery times. However, in the ablation and full transection experiments, many of the RS neurons were excessively swollen and could not be recorded from, yielding a relatively small sample size (see Results).

For RS neurons whose axons were injured at 10% BL and allowed to recover for 2 weeks, injury effects were maximal, and most neurons displayed bursting or high SFA firing patterns. However, not all neurons with an injured axon, as indicated by the absence of orthodromic responses below the lesion, displayed these firing patterns. Some RS neurons with injured axons displayed regular firing patterns. Perhaps after injury, the axons of RS neurons made synapses above the lesion that restored normal firing
properties. Therefore, one cannot predict the effects of injury of RS neuron based on injury to the axon alone, suggesting that the firing pattern of RS neurons is a better indicator of the degree of injury.

Previous studies indicate that a rostral spinal cord transection is a stronger stimulus for axonal regeneration of RS neurons than caudal transections (McClellan et al., 2005). Also, following rostral spinal cord transections, most axons of RS neurons regenerate for relatively short distances and presumably make synapses just below the lesion (Davis and McClellan, 1994a,b). Taken together, these results support the hypothesis that the degree to which a neuron is stimulated to regenerated is based, on part, on synapses it makes, either above or below a lesion (McClellan, 1998). In the present study, right hemi-transections at 10% BL caused a greater change in biophysical properties of RS neurons than did right hemi-transections at 30% or 50% BL. This suggests that the changes in biophysical properties of injured RS neurons described in the present study are indicative of a switch to a regenerative mode. Thus, the present study suggests that rostral spinal cord transections alter the biophysical properties of axotomized RS neurons to a greater degree than more caudal transections, and this may be causally linked to the greater axonal regeneration that follows rostral lesions compared to caudal injuries.

For the ablation type of injuries used in the present study, most neurons were not healthy enough to record intracellularly. For the RS neurons that were recorded from, almost half had orthodromic spikes above the lesion and about 75% had high SFA/bursting firing patterns. About 25% of these neurons had regular firing patterns, but perhaps these neurons had made synapses above the ablation site. For the experiments
with a full spinal cord transection at 10% BL and 8 week recovery times, most of the axons of RS neurons regenerated through the lesion site but still displayed injury-type firing properties. However, at this recovery time, only ~0-30% of the Müller cells that were examined in the present study (M2, M3, I1, B1, B3, and B4; see Fig. 1B) would have regenerated for ~10 mm below the lesion (Davis and McClellan, 1994b). Thus, many of these Muller cells might not have regenerated their axons for substantial distances below the lesion and made sufficient numbers of synapses to restore normal biophysical properties. Additional experiments will be needed to test these possibilities.

Relationship of Changes in Biophysical Properties and Axonal Regeneration

Following spinal cord injury, axotomized RS regenerate their axons across the injury site, make synapses with spinal neurons below the lesion, and restore locomotor function (McClellan, 1998). Since this axonal regeneration occurs during the recovery times that were used in the present study, it is important to consider whether the changes in biophysical properties of injured RS contribute to the ability of these neurons to extend their axons. In lamprey neurons, calcium influx during action potentials contributes substantially to the amplitude of the sAHP (Wikstrom and El Manira, 1998; Cangiano et al. 2002). In the present study in which RS neurons were axotomized at 10% BL and recovered for 2 weeks, the sAHP was absent or significantly reduced, possibly suggesting a down regulation of calcium channels and a reduction in calcium influx during neural activity. In neuronal cell culture, manipulations that elicit a local influx of calcium in the growth cones of RS neurons inhibit neurite outgrowth or cause neurite retraction (Hong et al, 2002; Ryan et al., 2004). Together, these results suggest that axotomized RS neurons may down-regulate calcium channels to maintain relatively low intracellular
calcium levels and promote axonal regeneration. In the future, it will be interesting to attempt to block some of the changes in biophysical properties of injured RS neurons to test whether these changes are critical for axonal regeneration.

Comparison to Other Lamprey Studies

In the lamprey, the effects of axotomy on dorsal cells (DCs), sensory neurons whose cell bodies are located in the spinal cord, has been examined (Yin et al., 1981). Approximately 4-5 weeks following spinal cord transection at ~20% BL, axotomized DCs had increases in input resistance, voltage threshold, spike width, spike overshoot, and conduction velocity, and decreases in the resting membrane potential and rate of rise of action potentials. Interestingly, these changes in properties were not dependent on the distance between the spinal cord transection and the somata of DCs. Also, these changes in biophysical properties lagged behind the morphological changes that occur in injured DCs. In contrast, in the present study most of the above biophysical properties did not changed after axotomy of RS neurons. The different results regarding responses of RS neurons and DCs to axotomy might be due to differences in function (descending vs. sensory) or size of these neurons. In addition, the different results might be due to differences in the state of technology at the time of the experiments. For example, the resting membrane potential of control dorsal cells was -56.4 ± 5.6 (Yin et al., 1981), while in the present study, RS neurons had resting potentials of -75.5 ± 4.72 mV. Also, the DCC mode for intracellular recording, which was used in the present study for accurate measurements of spike threshold and membrane resistance, was not available when the study on DCs was conducted.
Comparisons to Axotomy of the Mauthner Cells in Goldfish

At 0-120 days following axotomy 5-11 mm from the cell body of goldfish Mauthner cells, a RS neuron involved in escape responses, there was no change in the resting membrane potential, voltage threshold, or input resistance, but action potential amplitude increased (Titmus et al. 1986). In contrast, in the same animal model and neuron, Faber et al. (1981) reported an increase in input resistance. Thus, there appear to be some differences in the response of goldfish Mauthner cells and lamprey RS neurons to axotomy.

Comparisons to Axotomy of Neurons in Higher Vertebrates

Following axotomy of various types of neurons in higher vertebrates, changes in biophysical properties often are quite variable, and it is difficult to identify “typical” responses to injury. Below is a brief summary of changes in biophysical properties of neurons following axotomy in higher vertebrates that are similar to the changes found in the present study: (1) no change in resting membrane potential following axotomy of rat central nerves (Mandolesi et al., 2004; Chen and Tseng, 1997; Tseng and Prince, 1996), rat DRG (Abdulla and Smith, 2001; Stebbing et al., 1999; Zhang et al., 1997), and guinea pig, rat, and cat motoneurons (Laiwand et al., 1988; Umemiya et al., 1993; Kuno et al., 1974); (2) no change in action potential amplitude following axotomy of mouse trigeminal nerves (Cherkas et al., 2004), rat central nerves (Chen and Tseng, 1997; Tseng and Prince, 1996) or DRG (Stebbing et al., 1999) or motoneurons (Umemiya et al., 1993), and frog sympathetic nerves (Shapiro et al., 1987); and (3) no change in input resistance following cat motoneurons (Kuno et al., 1974). However, in other studies, some of these properties did change following axotomy (see Introduction).
In contrast to one of the main results from the present study, fAHPs did not change following axotomy of rat central neurons (Chen and Tseng, 1997), rat DRG neurons (Abdulla and Smith, 2001), and guinea pig motoneurons (Laiwand et al., 1988). Also, following axotomy of cat motoneurons (Yamuy et al., 1992) or rat DRG (Abdulla and Smith, 2001), sAHPs did not change. In general, the changes in biophysical properties of neurons in higher vertebrates are quite variable, and it is difficult to identify “typical” changes. Thus, there does not appear to be a universal, consistent response of neurons to axotomy, at least with regard to biophysical properties.

Future Studies

In the future, examining biophysical properties at longer recovery times than 6-8 weeks following right hemi-transections would allow a more detailed analysis of the time course of recovery of properties for axotomies at different levels of the spinal cord. In addition, extending the recovery times following spinal cord ablations and full spinal cord transections to time periods in which greater numbers of RS neurons regenerate their axons for substantial distances would clarify if connection with targets below the lesion are important for the recovery of normal biophysical properties. Furthermore, it would be worthwhile conducting anatomical studies to analyze the morphology of injured RS neurons and the presence of synaptic connections on these neurons in conjunction with the above studies on changes in biophysical properties at longer recovery times following axotomy.
Summary

The present study suggests that rostral spinal cord injuries are a stronger stimulus than caudal spinal cord injuries not only for axonal regeneration but also for changes in biophysical properties. At least some of these changes in biophysical properties may be more than just a consequence of injury but may be important in allowing RS neurons in the lamprey to regenerate their axons. If the specific changes in biophysical and molecular properties of RS neurons that promote axonal regeneration can be identified, it may be possible to induce these changes in neurons that presently are unable to regenerate in higher vertebrates, including humans.
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