Development/Plasticity/Repair

Spinal Cord Injury Induces Changes in Electrophysiological Properties and Ion Channel Expression of Reticulospinal Neurons in Larval Lamprey

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In larval lamprey, hemitransections were performed on the right side of the rostral spinal cord to axotomize ipsilateral reticulospinal (RS) neurons. First, at short recovery times (2–3 weeks), uninjured RS neurons contralateral to hemitransections fired a smooth train of action potentials in response to sustained depolarization, whereas axotomized neurons fired a single short burst or short repetitive bursts. For uninjured RS neurons, the afterpotentials of action potentials had three components: fast afterhyperpolarization (fAHP), afterdepolarizing potential (ADP), and slow AHP (sAHP) that was attributable to calcium influx via high-voltage-activated (HVA) (N- and P/Q-type) calcium channels and calcium-activated potassium channels (SKKCa). For axotomized RS neurons, the fAHP was significantly larger than for uninjured neurons, and the ADP and sAHP were absent or significantly reduced. Second, at relatively long recovery times (12–16 weeks), axotomized RS neurons displayed firing patterns and afterpotentials that were similar to those of uninjured neurons. Third, mRNA levels of lamprey HVA calcium and SKKCa channels in axotomized RS neurons were significantly reduced at short recovery times and restored at long recovery times. Fourth, blocking calcium channels in uninjured RS neurons resulted in altered firing patterns that resembled those produced by axotomy. We demonstrated previously that lamprey RS neurons in culture extend neurites, and calcium influx results in inhibition of neurite outgrowth or retraction. Together, these results suggest that the downregulation of Ca^{2+} channels in axotomized RS neurons, and the associated reduction in calcium influx, maintain intracellular calcium levels in a range that is permissive for axonal regeneration.

Key words: **regeneration; axotomy; calcium channels; afterhyperpolarization; injury; locomotion**

Introduction

Axotomy (i.e., axonal injury) elicits a number of electrophysiological, morphological, and molecular changes in injured neurons (Titmus and Faber, 1990). However, the changes in electrophysiological properties after axotomy are variable and depend on the type of neuron as well as the organism. For example, in cat and rat, injured dorsal root ganglia (DRG) neurons display a decrease in the amplitude and duration of the afterhyperpolarization (AHP) (Gallego et al., 1987; Sapunar et al., 2005), whereas the duration of AHPs in injured DRG neurons in hamster (Gurtu and Smith, 1988) or mouse (Liu et al., 2002) does not change.

Many axotomized neurons display changes in ion currents, channel conductances, and gene expression for ion channels. For axotomized mouse DRG neurons (Andre et al., 2003a) and injured rat sympathetic neurons (Sanchez-Vives and Gallego, 1994), there is an increase in calcium-activated chloride currents. In the sympathetic system of frogs, axotomized neurons exhibit a decrease in both calcium and calcium-activated potassium currents resulting in reduction of the slow component of the AHP (Jassar et al., 1993).

After severe spinal cord injury in higher vertebrates, such as birds and mammals, there is very limited axonal regeneration of axotomized neurons and usually no recovery of functions caudal to the lesion (for review, see Schwab and Bartholdi, 1996; Bradbury and McMahon, 2006). In contrast, spinal cord transection in several lower vertebrates, including lamprey, fish, and certain amphibians, leads to robust regeneration of axons through the injury site, formation of synaptic connections, and virtually complete behavioral recovery within a few weeks (for review, see McClellan, 1998).

In larval lamprey, reticulospinal (RS) neurons are both sufficient and necessary for initiation of locomotion (Paggett et al., 2004; Jackson et al., 2007). After a rostral spinal cord transection, axotomized RS neurons regenerate their axons for progressively greater distances caudal to the lesion with increasing recovery times (Davis and McClellan, 1994a,b), and there is a gradual recovery of locomotor function (Davis et al., 1993; McClellan, 1994) (for review, see McClellan, 1998). Our long-term goals are to determine the changes in properties of axotomized RS neurons after spinal cord injury and ultimately to assess whether these changes are important for axonal regeneration.

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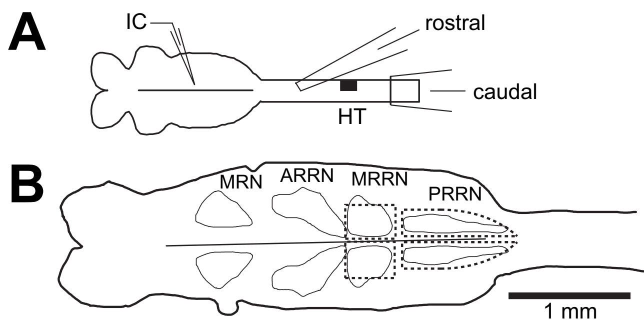

Figure 1. ***A***, Isolated brain/spinal cord preparation showing the brain (left) and rostral spinal cord, right spinal cord hemitransection (HT) at 10% body length (BL) (normalized distance from the anterior head), intracellular recording micropipette (IC), "rostral" suction electrode on the right, dorsal half of the spinal cord above the hemitransection, and "caudal" suction electrode around the spinal cord below the hemitransection. ***B***, Diagram of the lamprey brain showing reticular nuclei: MRN, ARRN, MRRN, and PRRN (Rovainen, 1979). Large identified RS Müller cells (B cells; B1, B3, and B4) used in parts of the present study are located in the MRRN. Dotted lines indicate tissue excised for PCR experiments (see Materials and Methods).

In the present study, larval lamprey received right hemitransections of the rostral spinal cord to selectively axotomize RS neurons on the right side of the brain, ipsilateral to spinal lesions. At short recovery times (2–3 weeks), axotomized RS neurons displayed substantial changes in firing patterns, a significant reduction in components of the action potential that are dependent on calcium channels and calcium-activated potassium channels and a significant reduction in mRNA for these two types of channels. At long recovery times (12–16 weeks), neurophysiological properties and mRNA levels for these channels returned to normal. These data are discussed with regard to our recent experiments on neurite outgrowth of lamprey RS neurons in culture (Ryan et al., 2007).

Some of the neurophysiological data have been presented previously in abstract form (McClellan et al., 2002, 2006; McClellan, 2003; Kovalenko et al., 2007).

Materials and Methods

Animal care. Larval sea lamprey [*Petromyzon marinus* (Pm)] were maintained in ~10 L aquaria at 23–25°C and used for both neurophysiological and molecular biology experiments. The procedures used in the present study were approved by the Animal Care and Use Committee at the University of Missouri.

Spinal cord hemitransections. Lamprey ($n = 70$ animals, ~90–120 mm) were anesthetized in tricaine methanosulphonate (~200 mg/L; MS222; Sigma, St. Louis, MO), and fine forceps and iridectomy scissors were used to make hemitransections (Fig. 1*A*, HT) on the right side of the spinal cord at 10% body length (normalized distance from the anterior head), ~2 mm caudal to the brain at segment ~2–3. In the lamprey, RS neurons are located in the mesencephalic reticular nucleus (MRN), as well as the anterior (ARRN), middle (MRRN), and posterior (PRRN) rhombencephalic reticular nuclei (Fig. 1*B*) (Rovainen, 1979). In the lamprey brain, large identified RS neurons, called "Müller cells," have long ipsilateral axons that project to the caudal end of the spinal cord (Rovainen, 1979; Davis and McClellan, 1994b). The largest Müller cells in the MRRN are "B cells" (B1, B3, and B4), which were a major focus of the present study. In the MRRN and PRRN, ~94 and ~86%, respectively, of smaller unidentified RS neurons have ipsilateral descending axons (Shaw et al., 2001). Thus, hemitransections on the right side of the spinal cord axotomized all or most of the above neurons on the right side of the brain, whereas these neurons on the left side were mostly uninjured. In contrast, RS neurons in the ARRN have mixed ipsilateral/contralateral projections (Shaw et al., 2001), whereas <5% of the total number of RS neurons are located in the MRN (Davis and McClellan, 1994a).

Some animals had short recovery times (2–3 weeks; $n = 50$ animals), before substantial axonal regeneration has occurred (Davis and McClellan, 1994a,b) and at which time axotomy-induced changes in neurophysiological properties of RS neurons are most pronounced (McClellan et al., 2002; McClellan, 2003). Other animals had relatively long recovery times (12–16 weeks; $n = 20$ animals), at which time many axotomized RS neurons have regenerated their axons for at least 10 mm below the lesion (Davis and McClellan, 1994b), made synapse with spinal neurons (Mackler and Selzer, 1987), and display properties similar to those of uninjured neurons (McClellan et al., 2002).

Intracellular recordings. After recovery, the brains and spinal cords, up to ~30% body length, were removed, as described previously (Rouse et al., 1998), and pinned dorsal side up in a neurophysiological recording chamber containing oxygenated lamprey Ringer's solution (6–9°C; pH 7.4) (McClellan, 1990). A rostral suction electrode was placed on the right, dorsal surface of the spinal cord approximately halfway between the brain and spinal hemitransection, and a caudal suction electrode was placed around the spinal cord an average of 4.2 ± 3.7 mm (mean ± SD; $n = 65$ animals) or an average of ~4.8 ± 4.2 segments below the hemitransections (Fig. 1*A*). Intracellular recordings using either current clamp ("bridge mode") or "discontinuous current clamp" (DCC) (f_s ~4–6 kHz) were made from uninjured and axotomized RS neurons with micropipettes filled with 5 M potassium acetate (~60–100 MΩ). Only neurons with resting membrane potentials (V_{rest}) equal to or more negative than −65 mV were analyzed. There was no indication that axotomized RS neurons tended to have less negative membrane potentials than uninjured neurons. The effects of axotomy were examined in large identified RS neurons in the MRRN (B cells; B1, B3, and B4), as well as unidentified RS neurons in the MRRN and PRRN (Fig. 1*B*). The different B cells displayed similar changes in properties in response to axotomy. In the DCC mode, depolarizing pulses (+1–10 nA, 2 s) were applied to examine the effects of axotomy on repetitive firing patterns. In bridge mode, short 1–10 ms, +10 nA pulses were applied to elicit action potentials that were compared in uninjured and axotomized RS neurons. All data were stored on tape (11 kHz sampling rate per channel; Neuro-Data DR890; Cygnus Technologies, Delaware Water Gap, PA), as well as acquired by custom data acquisition and analysis software.

The action potential amplitude as well as the peak of the fast AHP (fAHP) (more negative than V_{rest} in ~52% of uninjured B cells), afterdepolarizing potential (ADP) (more positive than V_{rest} in ~75% of uninjured B cells), and slow AHP (sAHP) (always negative) were measured relative to resting membrane potential. If the peak of the fAHP was above or the peak of the ADP was below the resting potential (see Figs. 3*C1*, 4*C1*), values were not measured for these parameters. Because the fAHP, ADP, and sAHP occur in rapid succession, variations in the peaks of the fAHP and ADP (above or below V_{rest}) probably reflect slight differences in the amplitudes and/or timing of the three components of the afterpotential.

Orthodromic responses elicited by RS neurons were recorded rostral and caudal to the hemitransections (Fig. 1*A*). At short recovery times (2–3 weeks), the lack of orthodromic responses caudal to the hemitransections was taken to indicate that a given RS neuron, usually on the right side of the brain, had been axotomized. At long recovery times (12–16 weeks), orthodromic responses caudal to healed hemitransections elicited by axotomized RS neurons on the right side of the brain were taken as an indication that a neuron had regenerated its axon below the lesion site, at least for a few millimeters. Previous studies (Mackler and Selzer, 1987) indicate that, 7–40 weeks after spinal cord transection, stimulation of single Müller cells elicits ~0.5–1.0 mV EPSPs in spinal neurons caudal to the lesion and does not elicit action potentials. In addition, the orthodromic responses are virtually identical before and after adding calcium channel blockers to the bath. Thus, these orthodromic responses, which were elicited at ~1 Hz, are not attributable to activation of and recording from postsynaptic spinal targets.

In one group of control animals that did not receive hemitransections ($n = 13$ animals), the pharmacology of the sAHP in uninjured RS neurons was examined with one of the following agents added to the bath: 2 μM ω-conotoxin MVIIC (Tocris Bioscience, Ellisville, MO) to block high-voltage-activated (HVA) (N- and P/Q-type) calcium channels (McDonough et al., 1996; Bussieres and El Manira, 1999) or 20 μM apamin

(Sigma) to block calcium-activated potassium (SKKCa) channels (Hill et al., 1992; Meer and Buchanan, 1992; Cangiano et al., 2002). Recordings were made at least 15 min after a drug was added to the bath. In a second group of control animals, firing patterns of uninjured RS neurons (n = 11 animals) were recorded before and after the addition of 2 mM $CoCl_2$ to the bath to block calcium influx and substantially reduce the sAHP to mimic the effects of axotomy (see Results).

Cloning lamprey ion channels. In the present study, it was found that the sAHP is mediated by calcium influx via HVA calcium channels and activation of SKKCa channels and that the sAHP was significantly decreased after axotomy. Thus, the decreased sAHP might be attributable to downregulation of HVA calcium channels, SKKCa channels, or both. To distinguish these possibilities, we conducted experiments to measure mRNA expression levels for these channels in reticular nuclei containing uninjured and axotomized RS neurons.

We cloned and sequenced partial open reading frames for α subunits from two ion channels from the sea lamprey, *Petromyzon marinus*: an SKKCa channel and an HVA calcium channel ($Ca_v2.x$; see explanation below). This was accomplished using reverse transcription (RT)-PCR with a combination of both degenerate and specific oligonucleotide primers.

Ion channels were cloned from larval sea lamprey using RT-PCR with degenerate primers based on conserved amino acid and nucleotide alignments from *Mus musculus*, *Rattus norvegicus*, *Homo sapiens*, *Danio rerio*, and, when available, *Tetraodon nigrividus.* cDNA template derived from total RNA extracted from nervous system tissue (brain and spinal cord) was used to generate PCR amplicons of predicted size based on amino acid alignments. These PCR products were cloned into pGem-T-easy plasmid vector (Promega, Madison, WI) and sequenced using dye terminator cycle sequencing (CEQ DTCS Quick Start Kit; Beckman Coulter, Fullerton, CA). Sequences obtained were compared with orthologous sequences using BlastX (National Center for Biotechnology Information). GenBank accession numbers for these sequences are as follows (for *Petromyzon marinus*): PmHVA calcium, EF585497; PmSKKCa, EF585498.

The predicted amino acid sequence of our partial PmSKKCa open reading frame shares 90% amino acid identity with both human and mouse SKKCa channels. The predicted amino acid sequence of our partial HVA calcium channel sequence shares 79% amino acid identity (88% positives) with both P/Q-type ($Ca_v2.1$) and N-type ($Ca_v2.2$) calcium channels in humans and mice. Therefore, although we are confident in the identity of this as a HVA calcium channel (N- and P/Q-type but not L-type), we have not yet determined to which subfamily of HVA calcium channels our gene belongs. This was acceptable for the present study because the sAHP, which was substantially reduced in axotomized neurons, is primarily dependent on calcium influx via HVA channels (see Fig. 3*D*). Throughout this study, we refer to this channel as HVA calcium channels or $PmCa_v2.x$.

Real-time PCR. Larval sea lamprey were anesthetized, spinal cord hemitransections were performed on the right side at 10% body length, and animals were allowed to recover for 1 week (n = 9 animals) or 11–17 weeks (n = 5 animals). A 1 week recovery time was used because it was assumed that molecular changes in ion channel expression would precede changes in biophysical properties, which are maximal at ~2–3 weeks (McClellan et al., 2002). After recovery, the brains and rostral spinal cords were removed in lamprey Ringer's solution, and iridectomy scissors were used to make dorsoventral cuts in the brain to remove the MRRN and PRRN on the left and right sides of the brain (Fig. 1*B*, dotted lines). In larval sea lamprey (mean length, 125 mm), there are ~80 (~360) RS neurons in the MRRN (PRRN) on each side of the brain (Davis and McClellan, 1994a; Shaw et al., 2001; Zhang et al., 2002).

Each individual brain region was placed directly into 350 μl of lysis buffer (buffer RLT as provided by Qiagen, Valencia, CA) containing 1% β-mercaptoethanol and frozen at −80°C until RNA extraction. Primers specific for real-time PCR detection of *PmSKKCa*, $PmCa_v2.x$, and 18S rRNA (based on published sequence for lamprey; GenBank accession number M97575) using SYBR Green were developed and designed using Primer3 software. The sequences of primers were as follows: (1) 18S forward, 5′- CGACCGTTGCTTGGATAACT-3′; 18S reverse, 5′- CCGGATTGGTTTTGGTCTAA-3′; PmSKKCa forward, 5′- CACACACCTACTGTGGACGG-3′; (2) PmSKKCa reverse, 5′- GTTTCTCAGCCTTGGTCAGC-3′; and (3) $PmCa_v2.x$ forward, 5′-ATTCTCGTCACGGAGCTTGG-3′; $PmCa_v2.x$ reverse, 5′-GGAGGATGCGGATGGTGTAG-3′.

Total RNA was isolated from individual nuclei using the RNeasy Micro kit (Qiagen, Valencia, CA) according to the instructions of the manufacturer, including an on-column DNase digestion to remove potential genomic DNA contamination. Reverse transcription was performed with SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) primed with a mix of both oligo-dT and random hexamers. cDNA was then purified via overnight ethanol precipitation and resuspended in nuclease-free water. Purified cDNA was used as a template in a PCR reaction in 1× Quantitect SYBR Green Master Mix (Qiagen) with 300 nM of each forward and reverse primers. PCR and fluorescence detection were performed in a RotorGene 3000 thermocycler (Corbett Research, Sydney, Australia) using 35–45 cycles as follows: 20 s at 95°C → 20 s at 58°C → 20 s at 72°C. Acquisition of SYBR Green fluorescence occurred at the 72°C step of the PCR and was analyzed with RotorGene 6 software (Corbett Research). Melt curves revealed only a single PCR product for any given reaction. Each reaction was performed in triplicate.

Care was taken at each step to minimize assay variability: samples were processed in parallel whenever possible, the same batch of reverse transcriptase was used for all samples, and PCR runs were designed to maximize the number of samples run in each batch. Fold difference in mRNA expression levels for HVA calcium or SKKCa channels between right and left nuclei in each animal then was calculated using the $\Delta\Delta$Ct method with 18S rRNA as the control gene for normalization (Livak, 1997).

Statistics. For 2–3 week recovery times, neurophysiological parameters were determined for axotomized B cells (B1, B3, and B4 cells in the MRRN), which included all right B cells as well as those left B cells that did not elicit an orthodromic response in the recording electrode caudal to the hemitransections. If the ADP and sAHP components were clearly absent from the action potential (see Fig. 3*B2*), they were assigned values of zero. In other cases in which it was unclear whether a component was present or it was problematic to measure (e.g., fAHP peak above V_{rest} in Fig. 4*C1*; ADP peak below V_{rest} in Fig. 3*C1*), values were omitted from the analysis. Thus, in Table 1, there are sometimes different n values for the different parameters. Also, for some neurons, only firing patterns or electrophysiological parameters of action potentials were determined. ANOVA or Kruskal–Wallis test was used to compare the electrophysiological parameters between the following sets of B cells (Table 1): (1) uninjured neurons (always on left side) and axotomized neurons at short recovery times; (2) right and left neurons at long recovery times; and (3) left or right neurons at long recovery times and uninjured neurons at short recovery times. For the molecular data, the ratios of the mean fold levels of expression for left nuclei (containing mostly uninjured RS neurons; see above) and right nuclei (containing mostly axotomized RS neurons) for each cell group were compared with 1.0 (i.e., equal expression levels) using a one-sample t test. Statistical significance was assumed when $p \leq 0.05$.

Results

Firing patterns of identified RS neurons

At short recovery times (2–3 weeks) after hemitransections of the right side of the rostral spinal cord (Fig. 1*A*) (see Materials and Methods), 70% of large, identified RS neurons in the MRRN on the left side of the brain (n = 39 of 56 left B cells) elicited orthodromic responses in the caudal recording electrode below the hemitransections (Fig. 1*A*) (see below). For these particular neurons, 95% fired smooth, regular trains of action potentials with moderate spike frequency adaptation in response to application of depolarizing current pulses (Fig. 2*A1*,*B1*), as described previously for virtually all uninjured B cells in animals without spinal cord lesions (Rouse et al., 1998). The right (n = 81 neurons) and left (n = 17 neurons) B cells that did not elicit orthodromic responses in the caudal electrode below the lesion (Fig. 3*B1*) and were considered

Table 1. Biophysical properties of B cells

	V_{rest}[e] (mV)	V_{AP}[f] (mV)	fAHP (mV)	ADP (mV)	sAHP (mV)
Short recovery times (2–3 weeks)					
Uninjured neurons[a]	−75.6 ± 5.0[g]	105.0 ± 7.7	−3.83 ± 2.43	2.96 ± 1.64	−1.80 ± 1.26
	(n = 31)[h]	(n = 30)	(n = 16)	(n = 23)	(n = 30)
Axotomized neurons[b]	−74.9 ± 4.9	106.8 ± 10.3	−9.46 ± 4.17***	0.11 ± 0.33***	−0.34 ± 0.65***
	(n = 98)	(n = 96)	(n = 82)	(n = 40)	(n = 78)
Long recovery times (12–16 weeks)					
Left neurons[c]	−72.7 ± 4.5	98.2 ± 11.1	−4.51 ± 2.27	1.92 ± 1.49	−1.41 ± 0.65
	(n = 13)	(n = 13)	(n = 11)	(n = 9)	(n = 13)
Right neurons[d]	−74.0 ± 5.4	103.2 ± 8.2	−2.10 ± 1.85	2.09 ± 1.35	−1.25 ± 0.59
	(n = 18)	(n = 17)	(n = 15)	(n = 16)	(n = 17)

n, Number of neurons. Statistics: ***$p \leq 0.001$ (ANOVA or Kruskal–Wallis test) parameters compared between uninjured and axotomized neurons (short recovery times), left and right neurons (long recovery times), and left or right neurons and uninjured neurons (see Materials and Methods).

[a]B cells (B1, B3, and B4 in MRRN; see Fig. 1*B*) on the left side of the brain that elicited orthodromic responses in the recording electrode caudal to spinal hemitransections (see Fig. 1*A*).

[b]B cells on the right side of the brain as well as those cells on the left side that did not elicit orthodromic responses caudal to the lesion.

[c]B cells on the left side of the brain, all of which elicited orthodromic responses in the recording electrode caudal to spinal hemitransections (see Fig. 1 *A*).

[d]B cells on the right side of the brain that elicited orthodromic responses caudal to the lesion.

[e]V_{rest}, Resting membrane potential.

[f]V_{AP}, Amplitude of action potentials.

[g]Mean ± SD.

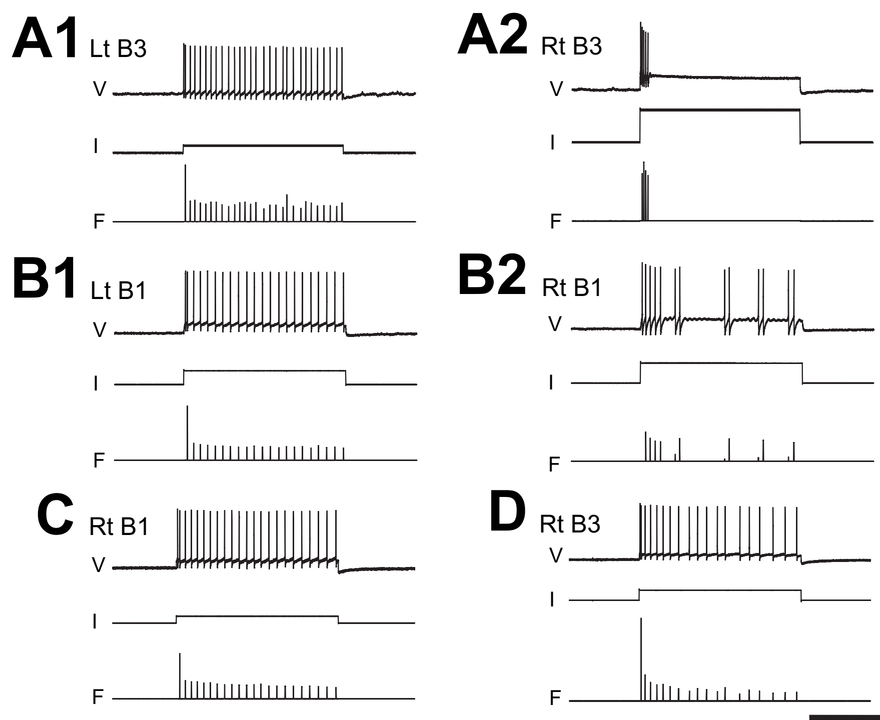

Figure 2. Firing patterns of uninjured and axotomized large identified RS neurons ("B" cells in the MRRN; see Fig. 1*B*). ***A***, ***B***, Short recovery times (2 weeks). Membrane potential (V), current (I), and instantaneous firing frequency (F). ***A1***, ***B1***, Uninjured B cells fired smooth, regular trains of action potentials in response to depolarizing current pulses. ***A2***, ***B2***, The same contralateral axotomized neurons in the same preparation displayed a single short burst or short multiple bursts. ***C***, ***D***, Long recovery times (12 weeks). Axotomized B cells on the right side of the brain fired smooth, regular trains of action potentials similar to those of uninjured RS neurons. Vertical calibration bar: 100 mV, 14 nA, 40 Hz; horizontal scale bar: 1 s. Rt, Right; Lt, left.

injured had the following firing patterns in response to maintained depolarization: (1) single short burst of action potentials with relatively high frequency and very high spike frequency adaptation (45% of neurons) (Fig. 2*A2*); (2) short, repetitive bursts (38%) (Fig. 2*B2*); (3) erratic firing in which the frequency of action potentials did not decline smoothly (12%; data not shown); and (4) smooth, regular train of action potentials (5%). In general, higher depolarizing currents and potentials often were needed to reach threshold and elicit action potentials in axotomized, identified RS neurons compared with uninjured neurons (McClellan et al., 2002).

At relatively long recovery times (12–16 weeks), all of the identified RS neurons on the left side of the brain (n = 13 B cells) elicited orthodromic responses caudal to the healed hemitransections, and, of these neurons, 92% displayed smooth, regular firing patterns (data not shown). For the axotomized, identified RS neurons on the right side of the brain, 68% (n = 13 of 19 B cells) elicited orthodromic responses caudal to the healed hemitransections, and, of these neurons, 85% displayed smooth, regular firing patterns in response to maintained depolarization (Fig. 2*C*,*D*), similar to that of uninjured neurons. Thus, some axotomized neurons that did not recover normal firing patterns appeared to regenerate their axons but perhaps not far enough to make substantial numbers of synapses below the lesion (see Discussion). For example, at relatively long recovery times (16 weeks) after complete transections of the rostral spinal cord, only ∼30–50% of the large B cells (B1, B3, and B4) regenerate their axons for at least ∼10 mm below the lesion (Davis and McClellan, 1994b) (also see Zhang and McClellan, 1999).

Action potentials of identified RS neurons

At short recovery times, the resting membrane potential and action potential amplitude were not significantly different in uninjured and axotomized identified RS neurons ($p > 0.05$, ANOVA) (Table 1). Furthermore, uninjured RS neurons evoked orthodromic responses in the spinal cord rostral and caudal to the lesion (Figs. 1*A*, 3*A1*), whereas axotomized RS neurons only evoked responses rostral to the lesion (Fig. 3*B1*). However, some

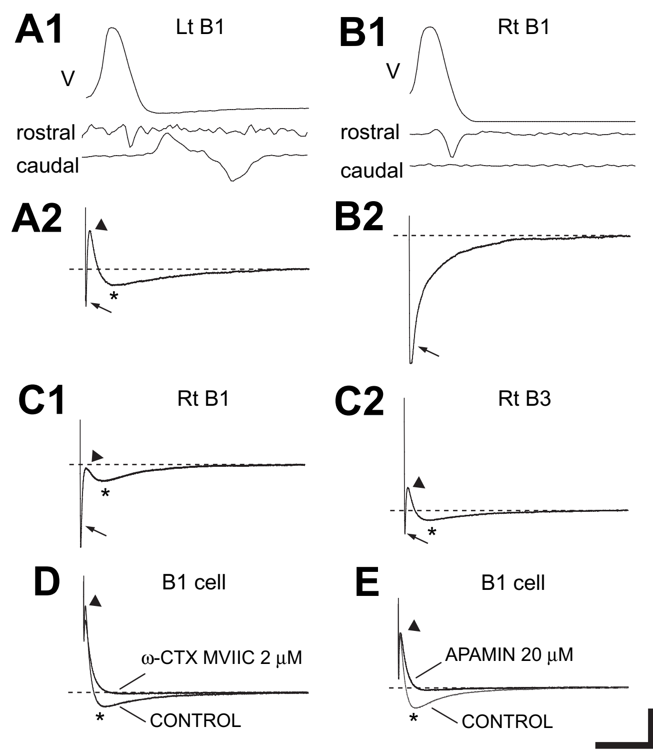

Figure 3. Action potential properties of uninjured and axotomized large identified RS neurons (B cells). ***A***, ***B***, Short recovery times (2 weeks). Uninjured (***A***) and axotomized (***B***) B cells in the same preparation. ***A1***, Action potential (V, single trace) in uninjured B cell elicited an orthodromic response in spinal recording electrodes rostral and caudal to the hemitransection (see Fig. 1*A*). ***B1***, Spikes in axotomized B cell evoked an orthodromic response only in the recording electrode rostral to the lesion. ***A2***, Action potentials in uninjured B cell included an afterpotential with three components (averaged traces in this and Fig. 4): fAHP (arrow), ADP (arrowhead), and sAHP (*). ***B2***, In an axotomized B cell, the fAHP (arrow) was relatively large, whereas the ADP and sAHP were absent. ***C***, Long recovery times (12 weeks). Axotomized B cells, both in the same preparation, displayed action potentials in which the afterpotential had three components, including an fAHP (arrow), ADP (arrowhead), and sAHP (*). ***D***, ***E***, Recordings from uninjured control B1 cells in brains from animals without spinal lesions. ***D***, The sAHP (control) was virtually abolished after application of 2 μM ω-conotoxin (CTX) MVIIC, but the fAHP and ADP appeared to be essentially unchanged. ***E***, The sAHP was almost completely blocked by 20 μM apamin. Vertical/horizontal calibration bars: ***A1***, ***B1***, 50 mV, 2.5 ms; ***A2***, ***B2***, ***C–E***, 3.5 mV, 100 ms.

axotomized neurons did not evoke orthodromic responses rostral to the lesion, suggesting that injured descending axons had retracted.

For uninjured RS neurons, the afterpotential of action potentials consisted of three components (Fig. 3*A2*): fAHP, ADP, and sAHP (see Materials and Methods). At 2–3 week recovery times after hemitransections, axotomized identified RS neurons displayed action potentials in which the fAHPs were significantly larger than those of uninjured neurons ($p \leq 0.001$, Kruskal–Wallis test) (Table 1; Fig. 3*B2*). In addition, the ADP and sAHP were absent or significantly decreased compared with those of uninjured neurons ($p \leq 0.001$, Kruskal–Wallis test) (Table 1; Fig. 3*B2*). Also, of the various electrophysiological parameters of the action potential that were measured, only the fAHP was significantly different (i.e., larger) in axotomized neurons that displayed a single short burst compared with those firing short repetitive bursts ($p = 0.049$, *t* test) (Fig. 2).

At long recovery times, 68% of identified axotomized RS neurons on the right side evoked orthodromic responses caudal to the healed spinal hemitransections (data not shown), indicative of descending axons that had regenerated at least a few millimeters. These particular neurons had afterpotentials with all three components (Fig. 3*C*), and 85% of the neurons displayed smooth, regular firing patterns in response to maintained depolarization (Fig. 2*C*,*D*). For right B cells that elicited orthodromic responses caudal to the hemitransections, the electrophysiological parameters were not significantly different from those in left B cells (Table 1). In addition, the electrophysiological parameters for right and left B cells at long recovery times (bottom of Table 1) were not significantly different from those of uninjured B cells at short recovery times (top of Table 1) ($p > 0.05$, ANOVA or Kruskal–Wallis test). In contrast, 50% of axotomized RS neurons ($n = 4$ total B cells) that recovered for relatively long times but did not elicit orthodromic responses caudal to spinal hemitransections, suggestive of failed axonal regeneration, displayed injury-type firing patterns (data not shown).

Pharmacology of the sAHP

Because the sAHP was absent or significantly decreased in axotomized identified RS neurons, the pharmacology of this component was examined in the brains of normal animals that had not received hemitransections. Blocking HVA (i.e., N- and P/Q-type) calcium channels with 2 μM ω-conotoxin MVIIC significantly reduced the sAHP to 2.5 ± 5.8% of control values ($n = 11$ neurons; $p \leq 0.001$, Wilcoxon's signed rank test) (Fig. 3*D*) but appeared to have little effect on the fAHP and ADP. In addition, blocking SKKCa channels with 20 μM apamin significantly reduced the sAHP to 8.1 ± 14.1% of control values ($n = 11$; $p \leq 0.0001$, one-sample *t* test) (Fig. 3*E*). Thus, the sAHP in RS neurons in larval lamprey appears to be attributable to calcium influx primarily via HVA (i.e., N- and P/Q-type) calcium channels and activation of calcium-activated potassium channels (SKKCa channels).

Neurophysiological properties of unidentified RS neurons

The PCR experiments below were conducted on relatively small pieces of brain tissue that included the entire MRRN or PRRN (Fig. 1*B*, dotted lines), which mostly contain relatively small, unidentified RS neurons (Davis and McClellan, 1994a,b). Therefore, neurophysiological experiments were conducted to determine whether the properties of uninjured and axotomized small unidentified RS neurons ($n = 32$ total neurons) were similar to those of large identified RS neurons (B cells; B1, B3, and B4). At 2–3 weeks after rostral spinal hemitransections, small uninjured RS neurons in the left MRRN and PRRN fired a smooth train of action potentials in response to maintained depolarization (data not shown), as was the case for uninjured B cells. In addition, action potentials of uninjured MRRN and PRRN neurons had afterpotentials that included an fAHP, ADP, and sAHP (Fig. 4*C1*,*D1*), much like those of large, identified RS neurons (i.e., B cells). Small axotomized RS neurons in the right MRRN and PRRN displayed altered firing patterns (Fig. 4*A*,*B*) comparable with those observed in axotomized B cells. For axotomized MRRN and PRRN neurons, the fAHP was significantly larger ($p \leq 0.05$, unpaired *t* test), whereas the ADP and sAHP were absent or significantly reduced ($p \leq 0.05$, Mann–Whitney *U* test) (Fig. 4*C2*,*D2*) compared with those for uninjured neurons. Thus, the effects of axotomy on unidentified RS neurons in the MRRN and PRRN were similar to those of large identified B cells.

Axotomy-induced changes in HVA Ca^{2+} and SKKCa channel expression

At short recovery times (1 week) after hemitransections on the right side of the rostral spinal cord, the mRNA expression levels

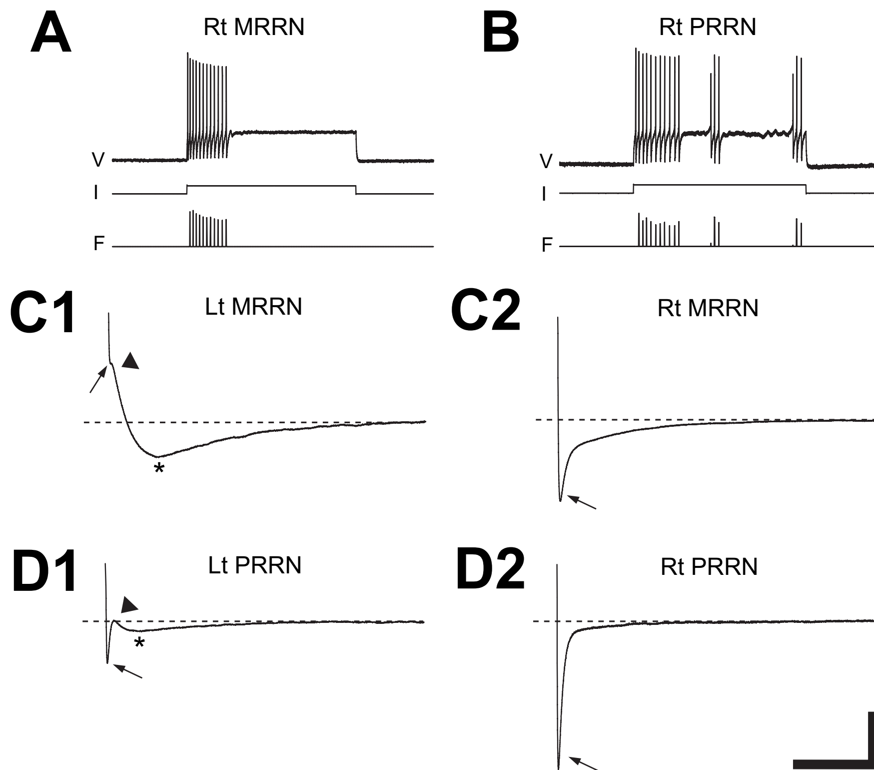

Figure 4. Properties of unidentified RS neurons in the MRRN and PRRN at 2 week recovery times after hemitransections at 10% body length (recordings from the same preparation). ***A***, ***B***, Unidentified axotomized RS neurons in the MRRN (***A***) and PRRN (***B***) had altered firing properties similar to those of axotomized B cells (see Fig. 2). ***C***, ***D***, Uninjured RS neurons in the MRRN (***C1***) or PRRN (***D1***) displayed action potentials that included an afterpotential with three components: fAHP (arrow), ADP (arrowhead), and sAHP (*). In axotomized MRRN (***C2***) and PRRN (***D2***) neurons, the fAHP was relatively large, whereas the ADP and sAHP were absent or substantially reduced (see Results). Vertical/horizontal calibration bars: ***A***, ***B***, 50 mV, 14 nA, 40 Hz/1 s; ***C***, ***D***, 3.5 mV/100 ms. Rt, Right; Lt, left.

for HVA calcium ($Ca_v2.x$) and SKKCa channels were significantly lower in both the MRRN and PRRN ipsilateral to the lesion (Fig. 5). SKKCa expression levels in the nuclei on the right side were approximately half of those on the left side in both the MRRN and PRRN (43 and 53%, respectively). Similar results were obtained for HVA calcium channel expression, which was reduced in right MRRN and PRRN to 58 and 43%, respectively, of that in left nuclei. At long recovery times (11–17 weeks) after spinal hemitransections, mRNA levels for both HVA calcium and SKKCa channels recovered, and there were no significant differences in expression levels between the right and left sides of the brain in the MRRN or PRRN (Fig. 5). These results suggest that, at short recovery times, the absence or substantial reduction of the sAHP in axotomized RS neurons was attributable to downregulation of both HVA calcium and SKKCa channels. In the future, it will be important to correlate the biophysical properties and mRNA levels for various channels in individual RS neurons.

Blocking calcium channels in uninjured RS neurons

A significant decrease in the sAHP was one of the main effects of axotomy of lamprey RS neurons, and molecular data suggest a downregulation of both HVA calcium and SKKCa channels. We mimicked these effects of axotomy in uninjured RS neurons (B cells) in animals without hemitransections by applying cobalt ($n = 28$ total neurons), which blocked calcium influx. The resting membrane potential before (75.3 ± 5.5 mV) and after (73.7 ± 5.1 mV) cobalt was not significantly different ($p > 0.05$, paired *t* test), but cobalt significantly reduced the sAHP to 8.8 ± 13.2% of control values ($p \leq 0.0001$, paired *t* test; $n = 24$). In control recordings before cobalt application, uninjured RS fired a continuous train of action potentials during depolarizing potentials just above threshold (Fig. 6*A1*), and the frequency increased with increasing depolarization (Fig. 6*A2*,*A3*). In contrast, after application of 2 mM $CoCl_2$ to the bath, neurons fired one or two action potentials at membrane potentials just above threshold (Fig. 6*B1*). The minimum amplitude of depolarization required to elicit action potentials in the presence of cobalt was significantly increased by a factor of 1.9 ± 1.3 times that in control recordings ($p \leq 0.001$, one-sample *t* test; $n = 28$). Furthermore, in the presence of cobalt, increasing the amplitude of depolarization above threshold still resulted in a relatively short burst of action potentials that terminated before the end of the 2 s current pulse (Fig. 6*B2*,*B3*), similar to that for axotomized neurons (Fig. 2*A2*). In the presence of cobalt, depolarizing pulses applied to different uninjured RS neurons elicited short single bursts (74%), short repetitive bursts (7%) (Fig. 6*C1*,*D1*), erratic firing (11%) (data not shown), or smooth firing (7%). Thus, blocking calcium channels with cobalt in uninjured RS neurons usually resulted in firing patterns that resembled, at least in some respects, those produced by axotomy.

Discussion

Axotomy of reticulospinal neurons in larval lamprey

At short recovery times (2–3 weeks) after hemitransections of the rostral spinal cord, uninjured RS neurons elicited orthodromic responses caudal to the lesion and fired smooth trains of action potentials, similar to control RS neurons in brains from animals without spinal lesions (Rouse et al., 1998). Thus, uninjured RS neurons did not display injury-type firing patterns simply because of close proximity to axotomized neurons on the opposite side of the brain (Ma et al., 2003; Sarantopoulos et al., 2007). In the present study, the afterpotentials of action potentials in uninjured RS neurons had three components: fAHP, ADP, and sAHP that was mediated by HVA calcium and SKKCa channels. Thus, sAHPs in RS neurons in larval lamprey appear to be attributable to similar ion channels as those for spinal neurons in adult lamprey (Hill et al., 1992; El Manira and Bussieres, 1997; Wikström and El Manira, 1998). Axotomized RS neurons fired a single short burst of action potentials or short repetitive bursts during depolarization. The ADP and sAHP were absent or significantly reduced in axotomized RS neurons, whereas the fAHP was significantly larger than in uninjured neurons, but this may have been attributable, in part, to the absent or reduced ADP.

The ADP, which is not attributable to calcium channels (Fig. 3*D*) but might be mediated by slowly inactivating sodium channels (Hu et al., 2002), was substantially reduced after axotomy, but the mechanisms and functional consequences are unclear because, in lamprey neurons, the ADP has not been examined in

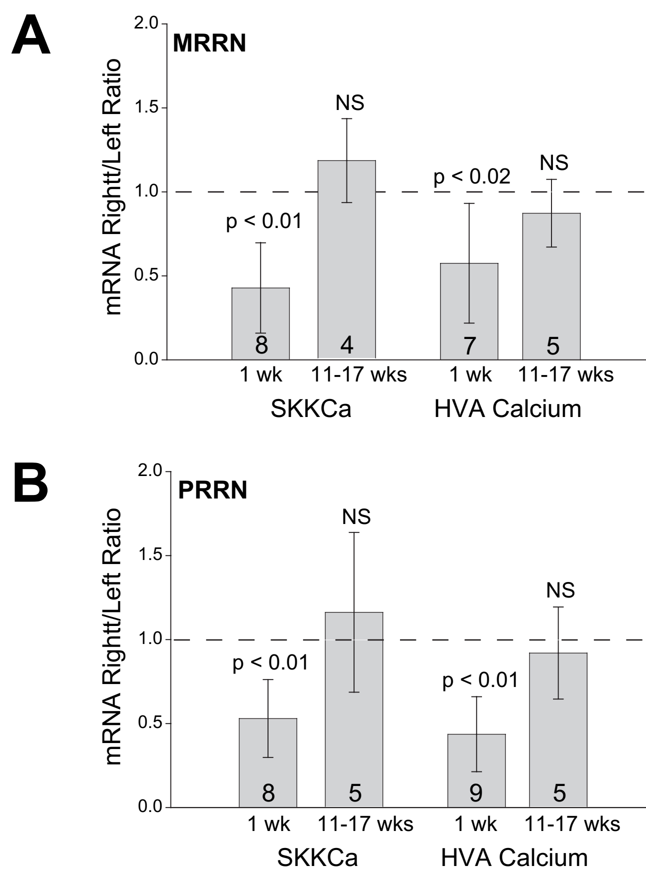

Figure 5. Relative mRNA expression levels for SKKCa and HVA calcium channels in reticular nuclei (MRRN and PRRN) after hemitransections at 10% body length. Bars indicate the mean ± SD fold difference of expression between reticular nuclei on the right (ipsilateral to transection) and left (control) sides of the brain (see Materials and Methods). Sample sizes indicated in each bar. The dashed line represents the level at which no difference in mRNA expression levels between right and left sides would be detected (i.e., ratio of 1.0). Significance values represent one-sample *t* tests with the test value set to 1.0. NS, Not significant.

detail. There are at least two explanations for the substantial decrease of both HVA calcium and SKKCa channels in axotomized RS neurons. First, perhaps the expression of these two channels is normally linked (Schulz et al., 2006), and this linkage is retained after axotomy. Second, the reduction in SKKCa channels might ensure that the sAHP is absent or substantially reduced even if some calcium influx remains through calcium channels or if calcium is released from internal stores.

At short recovery times, the absence of orthodromic responses caudal to hemitransections was used to indicate that the axons of RS neurons had been severed, whereas the presence of orthodromic responses at long recovery times was used to indicate that the axons of axotomized RS neurons had regenerated. However, at short recovery times, ~5% of axotomized RS neurons that did not elicit orthodromic responses caudal to hemitransections had smooth firing patterns. These few neurons may have not fully responded to axotomy, perhaps because their axons sealed very quickly and had limited injury current. Also, ~5% of left RS neurons that evoked orthodromic responses caudal to the lesion displayed injury-type firing patterns. Because in animals without spinal lesions, virtually all B cells fire smooth bursts of action potentials (Rouse et al., 1998), in the present study the axons of these left RS neurons might have been partially or transiently injured by the hemitransections but not sufficiently to disrupt the continuity of the descending projections. At long recovery times, ~8% of RS neurons on the right side of the brain that elicited orthodromic responses caudal to hemitransections had injury-type firing patterns. Perhaps these RS neurons regenerated their axons for short distances and did not make appropriate or sufficient numbers of synapses caudal to the lesion. For example, in other animals, disconnection of neurons from their synaptic targets can trigger changes in neurophysiological properties, and reconnection with these targets restores normal properties (Kuno et al., 1974; Foehring et al., 1986; Belmonte et al., 1988; Kelly et al., 1988; Pinter and Vanden Noven, 1989; Petrov et al., 2001). Unfortunately, in the lamprey, paired recordings between Müller cells and spinal neurons have a relatively low yield after spinal cord injury (Mackler and Selzer, 1987), and negative results are difficult to interpret.

In axotomized RS neurons, it is not presently known whether changes in components of the action potential (e.g., fAHP, ADP, and sAHP) and their underlying conductances account for the altered firing patterns. However, blocking calcium channels of uninjured RS neurons results in firing patterns that resembled those produced by axotomy (Fig. 6) (McClellan, 2003; McClellan et al., 2006). Also, in our computer model, eliminating the ADP and sAHP converted firing patterns from smooth, continuous to single short bursts, similar to one of the firing patterns of axotomized RS neurons (Kovalenko et al., 2007). A substantial reduction in the sAHP of axotomized RS neurons very likely results in the relatively high-frequency firing of action potentials observed for axotomized RS neurons (Fig. 2*A2*) (Wallen et al., 1989; Wikström and El Manira, 1998).

At early recovery times (1 week), there was a significant reduction of mRNA levels for both HVA calcium and SKKCa channels in the MRRN and PRRN ipsilateral to hemitransections relative to contralateral nuclei. Thus, the substantially decreased sAHPs in axotomized RS neurons (Fig. 3*B2*) appear to be attributable to reduction in expression of both these channels. However, HVA calcium and SKKCa channel mRNAs in the entire MRRN/PRRN were downregulated by only ~50% (Fig. 6). This apparent difference might be attributable to translational or posttranslational mechanisms that reduce the numbers/conductances of these channels. Also, there may have been some "contamination" in the MRRN/PRRN tissue samples from non-RS neurons or glial cells. In addition, unlike B cells that all have ipsilateral descending axons, ~6% of RS neurons in the MRRN and ~14% in the PRRN have contralateral projecting axons (Shaw et al., 2001). At long recovery times (11–17 weeks), expression levels for the above two channels were not significantly different in the right and left MRRN/PRRN.

Other lamprey studies

In larval lamprey, spinal cord transections elicit a number of electrophysiological changes in spinal cord dorsal cells (Yin et al., 1981), which are centrally located primary sensory neurons with ascending axons (Rovainen, 1979). For example, there is a reduction in resting membrane potential and maximal rate of rise of action potentials and an increase in spike threshold, spike width, spike overshoot, and conduction velocity (Yin et al., 1981).

Comparison with other vertebrate studies

In neurons of other vertebrates, axotomy can elicit changes in biophysical properties that are similar to those described here. In axotomized rat corticospinal neurons (Tseng and Prince, 1996), there is a decrease in the sAHP. In bullfrog sympathetic neurons, axotomy decreases the amplitude and duration of the sAHP

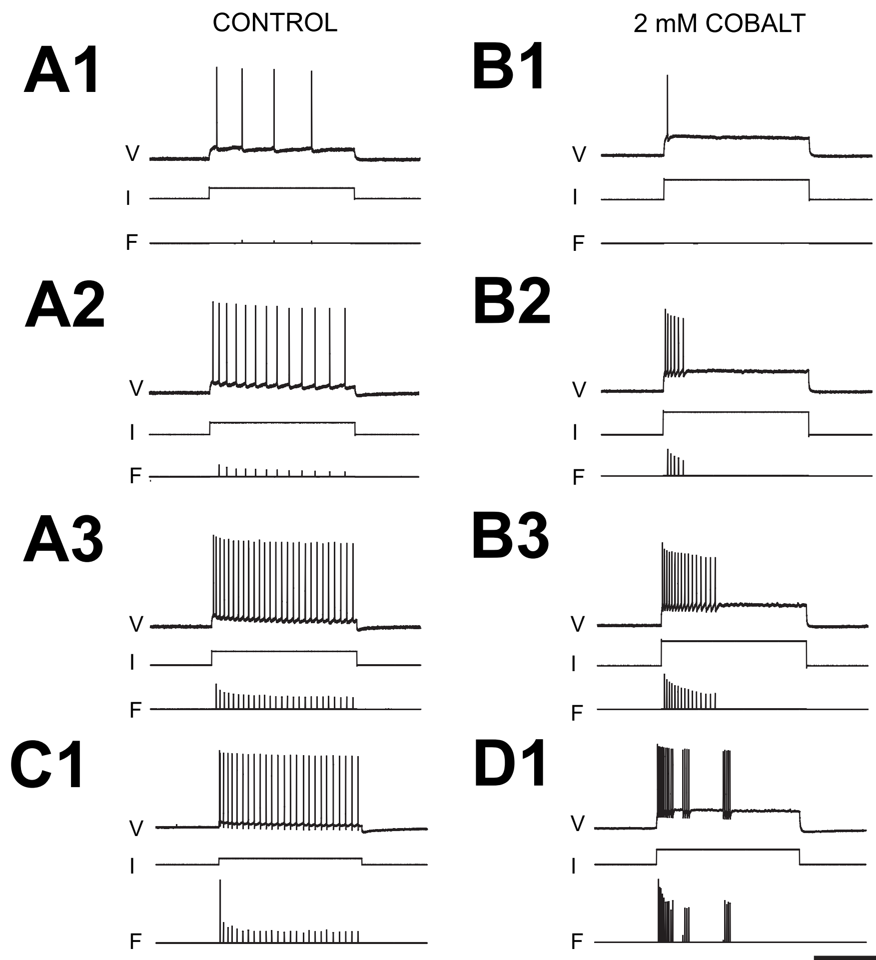

Figure 6. Blocking calcium channels produces injury-type firing properties in uninjured RS neurons from animals without hemitransections. ***A***, Uninjured left B3 cell in Ringer's solution (control). At membrane potentials just above threshold, the neuron fired a smooth continuous train of action potentials during the depolarizing pulse (***A1***) and with increasing depolarization fired with increasing frequency (***A2***, ***A3***). ***B***, Same uninjured B3 cell in ***A*** ~60 min after application of 2 mM $CoCl_2$ to the bath. The neuron fired a single action potential at membrane potentials just above threshold (***B1***) and with increasing depolarization fired only a relatively short burst, similar to axotomized RS neurons (***B2***, ***B3***) (see Fig. 2*A2*). Note that the threshold for producing action potentials was elevated after application of cobalt (see Results). ***C1***, ***D1***, An uninjured left B1 cell (different brain than in ***A***, ***B***) fired a smooth train of action potentials in Ringer's solution (control) (***C1***) and fired with short repetitive bursts after application of 2 mM cobalt (***D1***). Vertical calibration bar: 50 mV, 8.5 nA, 40 Hz; horizontal calibration bar: 1 s. V, Membrane potential; I, current; F, instantaneous firing frequency.

(Gordon et al., 1987; Kelly et al., 1986, 1988; Petrov et al., 2001), which appears to be attributable to a decrease in currents through both calcium and calcium-activated potassium channels (Kelly et al., 1986; Jassar et al., 1993). After axonal regeneration and reinnervation of peripheral targets, some of the normal features of action potentials of sympathetic neurons, such as the sAHP duration, return to normal (Kelly et al., 1988).

Axotomy-induced changes in neuron properties and axonal regeneration

In axotomy studies, a number of electrophysiological properties change in injured neurons, most of which are able to regenerate their peripheral axons (e.g., sensory, motor, and sympathetic neurons). However, little or no attention has been given to whether these changes are important for axonal regeneration. For most neuron types in culture, there appears to be a range of intracellular calcium concentrations that promotes neurite outgrowth, a phenomenon described by the "calcium set-point hypothesis" (for review, see Kater et al., 1988; Kater and Mills, 1991; Gomez and Spitzer, 2000; Henley and Poo, 2004). In particular, increases in intracellular calcium above this range inhibit neurite outgrowth. For example, larval lamprey RS neurons in culture extend neurites, and manipulations that induce calcium influx inhibit neurite outgrowth or elicit retraction (Hong et al., 2002; Ryan et al., 2004, 2007). In other preparations, contact of growth cones from cultured mammalian neurons with mature oligodendrocytes, which normally inhibit axonal regeneration after spinal cord injury (Schwab and Bartholdi, 1996), induces calcium influx and growth cone collapse (Bandtlow et al., 1993), which is prevented by calcium channel blockers (Moorman and Hume, 1993). Thus, for some neurons, intracellular calcium levels may be an important regulator of axonal regeneration after neuronal injury (McClellan, 2003; McClellan et al., 2006; Ryan et al., 2007). Interestingly, in many axotomy studies in which calcium channels have been examined, there is a reduction in calcium currents, conductances, and/or channel expression levels (Jassar et al., 1993; Baccei and Kocsis, 2000; Abdulla and Smith, 2001; Kim et al., 2001; Petrov et al., 2001; Andre et al., 2003b).

Conclusion

In larval lamprey, axotomized RS neurons displayed dramatic changes in their firing patterns, significant changes in components of the action potential (fAHP, ADP, and sAHP), and a significant reduction in mRNA levels for HVA calcium and SKKCa channels. These changes in neurophysiological properties and ion channel expression are a consequence of injury but may also be critical for subsequent axonal regeneration. After recovery, axotomized RS neurons might restore their normal neurophysiological properties with either the normal complement of ion channels present in uninjured neurons or different functionally equivalent proportions of ion channels (Marder and Prinz, 2002; Schulz et al., 2006). Experiments are in progress to test these and other hypotheses.

References

Abdulla FA, Smith PA (2001) Axotomy- and autotomy-induced changes in Ca^{2+} and K^{+} channel currents of rat dorsal root ganglion neurons. J Neurophysiol 85:644–658.

Andre S, Boukhaddaoui H, Campo B, Al-Jumaily M, Mayeux V, Greuet D, Valmier J, Scamps F (2003a) Axotomy-induced expression of calcium-activated chloride current in subpopulations of mouse dorsal root ganglion neurons. J Neurophysiol 90:3764–3773.

Andre S, Puech-Mallie S, Desmadryl G, Valmier J, Scamps F (2003b) Axotomy differentially regulates voltage-gated calcium currents in mice sensory neurons. NeuroReport 14:147–150.

Baccei ML, Kocsis JD (2000) Voltage-gated calcium currents in axotomized adult rat cutaneous afferent neurons. J Neurophysiol 83:2227–2238.

Bandtlow CE, Schmidt MF, Hassinger TD, Schwab ME, Kater SB (1993) Role of intracellular calcium in NI-35 evoked collapse of neuronal growth cones. Science 259:80 – 83.

Belmonte C, Gallego R, Morales A (1988) Membrane properties of primary sensory neurones of the cat after peripheral reinnervation. J Physiol (Lond) 405:219 –232.

Bradbury EJ, McMahon SB (2006) Spinal cord repair strategies: why do they work? Nat Rev Neurosci 7:644 – 653.

Bussieres N, El Manira A (1999) GABA(B) receptor activation inhibits N- and P/Q-type calcium channels in cultured lamprey sensory neurons. Brain Res 847:175–185.

Cangiano L, Wallen P, Grillner S (2002) Role of apamin-sensitive K_{Ca} channels for reticulospinal synaptic transmission to motoneuron and for the afterhyperpolarization. J Neurophysiol 88:289 –299.

Davis GR, McClellan AD (1994a) Extent and time course of restoration of descending brainstem projections in spinal-transected lamprey. J Comp Neurol 344:65– 82.

Davis GR, McClellan AD (1994b) Long distance axonal regeneration of identified lamprey reticulospinal neurons. Exp Neurol 127:94 –105.

Davis GR, Troxel MT, Kohler VJ, Grossmann EM, McClellan AD (1993) Time course of locomotor recovery and functional regeneration in spinal-transected lamprey: kinematics and electromyography. Exp Brain Res 97:83–95.

El Manira A, Bussieres N (1997) Calcium channel subtypes in lamprey sensory and motor neurons. J Neurophysiol 78:1334 –1340.

Foehring RC, Sypert GW, Munson JB (1986) Properties of self-reinnervated motor units of medial gastrocnemius of cat. II. Axotomized motoneurons and time course of recovery. J Neurophysiol 55:947–965.

Gallego R, Ivorra I, Morales A (1987) Effects of central or peripheral axotomy on membrane properties of sensory neurones in the petrosal ganglion of the cat. J Physiol (Lond) 391:39 –56.

Gomez TM, Spitzer NC (2000) Regulation of growth cone behavior by calcium: new dynamics to earlier perspectives. J Neurobiol 44:174 –183.

Gordon T, Kelly ME, Sanders EJ, Shapiro J, Smith PA (1987) The effects of axotomy on bullfrog sympathetic neurones. J Physiol (Lond) 392:213–229.

Gurtu S, Smith PA (1988) Electrophysiological characteristics of hamster dorsal root ganglion cells and their response to axotomy. J Neurophysiol 59:408 – 423.

Henley J, Poo MM (2004) Guiding neuronal growth cones using Ca^{2+} signals. Trends Cell Biol 14:320 –330.

Hill RH, Matsushima T, Shotland J, Grillner S (1992) Apamin blocks the slow AHP in lamprey and delays termination of locomotor bursts. NeuroReport 3:943–945.

Hong S-K, Groat CR, Nehra D, Shotts L, Armstrong JA, McClellan, AD (2002) Diffusible and non-diffusible factors that affect neurite outgrowth of descending brain neurons in culture from larval lamprey. Soc Neurosci Abstr 28:635.10.

Hu GY, Biró Z, Hill RH, Grillner S (2002) Intracellular QX-314 causes depression of membrane potential oscillations in lamprey spinal neurons during fictive locomotion. J Neurophysiol 87:2676 –2683.

Jackson AW, Pino FA, Wiebe ED, McClellan AD (2007) Movements and muscle activity initiated by brain locomotor areas in semi-intact preparations from larval lamprey. J Neurophysiol 97:3229 –3241.

Jassar BS, Pennefather PS, Smith PA (1993) Changes in sodium and calcium channel activity following axotomy of B-cells in bullfrog sympathetic ganglion. J Physiol (Lond) 472:203–231.

Kater SB, Mills LR (1991) Regulation of growth cone behavior by calcium. J Neurosci 11:891– 899.

Kater SB, Mattson MP, Cohan CS, Connor J (1988) Calcium regulation of the neuronal growth cone. Trends Neurosci 11:315–321.

Kelly ME, Gordon T, Shapiro J, Smith PA (1986) Axotomy affects calcium-sensitive potassium conductance in sympathetic neurones. Neurosci Lett 67:163–168.

Kelly ME, Bisby MA, Lukowiak K (1988) Regeneration restores some of the altered electrical properties of axotomized bullfrog B-cells. J Neurobiol 19:357–372.

Kim DS, Yoon CH, Lee SJ, Park SY, Yoo HJ, Cho HJ (2001) Changes in voltage-gated calcium channel α1 gene expression in rat dorsal root ganglia following peripheral nerve injury. Mol Brain Res 96:151–156.

Kovalenko M, Schulz DJ, McClellan AD (2007) Spinal cord injury induces changes in ion channels of reticulospinal neurons in larval lamprey. Soc Neurosci Abstr 33:137.17.

Kuno M, Miyata Y, Munoz-Martinez J (1974) Differential reaction of fast and slow α-motoneurones to axotomy. J Physiol (Lond) 240:725–739.

Liu C, Devor M, Waxman SG, Kocsis JD (2002) Subthreshold oscillations induced by spinal nerve injury in dissociated muscle and cutaneous afferents of mouse DRG. J Neurophysiol 87:2009 –2017.

Livak KJ (1997) Comparative Ct method. ABI Prism 7700 Sequence Detection System. User Bulletin 2. PE Applied Biosystems: Foster City, CA.

Ma C, Shu Y, Zheng Z, Chen Y, Yao H, Greenquist KW, White FA, LaMotte RH (2003) Similar electrophysiological changes in axotomized and neighboring intact dorsal root ganglion neurons. J Neurophysiol 89:1588 –1602.

Mackler SA, Selzer ME (1987) Specificity of synaptic regeneration in the spinal cord of the larval sea lamprey. J Physiol (Lond) 388:183–198.

Marder E, Prinz AA (2002) Modeling stability in neuron and network function: the role of activity in homeostasis. BioEssays 24:1145–1154.

McClellan AD (1990) Locomotor recovery in spinal-transected lampreys. Role of functional regeneration of descending axons from brainstem locomotor command neurons. Neuroscience 37:781–798.

McClellan AD (1994) Time course of locomotor recovery and functional regeneration in spinal-transected lamprey: In vitro brain/spinal cord preparations. J Neurophysiol 72:847– 860.

McClellan AD (1998) Spinal cord injury: lessons from locomotor recovery and axonal regeneration in lower vertebrates. Neuroscientist 4:250 –263.

McClellan AD (2003) Axotomy alters spike frequency adaptation of reticulospinal neurons in larval lamprey. Soc Neurosci Abstr 29:42.21.

McClellan AD, Hayter P, Chang Y-W (2002) Effects of axotomy on biophysical properties of reticulospinal neurons in larval lamprey. Soc Neurosci Abstr 28:635.12.

McClellan AD, Benes JA, Kovalenko M (2006) Axotomy-induced changes in the properties of reticulospinal neurons in larval lamprey. Soc Neurosci Abstr 32:323.14.

McDonough SI, Swartz KJ, Mintz IM, Boland LM, Bean BP (1996) Inhibition of calcium channels in rat central and peripheral neurons by ω-contotoxin MVIIC. J Neurosci 16:2612–2623.

Meer DP, Buchanan JT (1992) Apamin reduces the late afterhyperpolarization of lamprey spinal neurons, with little effect on fictive swimming. Neurosci Lett 143:1– 4.

Moorman SJ, Hume RI (1993) ω-Conotoxin prevents myelin-evoked growth cone collapse in neonatal rat locus ceruleus neurons *in vitro*. J Neurosci 13:4727– 4736.

Paggett KC, Jackson AW, McClellan AD (2004) Organization of higher-order brain areas that initiate locomotor activity in larval lamprey. Neuroscience 125:25–33.

Petrov T, Shapiro Y, Baker C, Duff JP, Sanders EJ, Gordon T, Smith PA (2001) Peripheral target contact regulates Ca^{2+} channels in the cell bodies of bullfrog sympathetic ganglion B-neurons. Auton Neurosci 89:74 – 85.

Pinter MJ, Vanden Noven S (1989) Effects of preventing reinnervation on axotomized spinal motoneurons in the cat. I. Motoneuron electrical properties. J Neurophysiol 62:311–324.

Rouse DT, Quan X, McClellan AD (1998) Biophysical properties of reticulospinal neurons in larval lamprey. Brain Res 779:301–308.

Rovainen CM (1979) Neurobiology of lamprey. Physiol Rev 59:1007–1077.

Ryan SK, Shotts L, Marsh G, McClellan AD (2004) Glutamate regulates neurite outgrowth of descending brain neurons in culture from larval lamprey. Soc Neurosci Abstr 30:729.6.

Ryan SK, Shotts L, Hong S-K, Nehra D, Groat C, Armstrong J, McClellan AD (2007) Glutamate regulates neurite outgrowth of cultured descending brain neurons from larval lamprey. Dev Neurobiol 67:173–188.

Sanchez-Vives MV, Gallego R (1994) Calcium-dependent chloride current induced by axotomy in rat sympathetic neurons. J Physiol (Lond) 475:391– 400.

Sapunar D, Ljubkovic M, Lirk P, McCallum JB, Hogan QH (2005) Distinct membrane effects of spinal nerve ligation on injured and adjacent dorsal root ganglion neurons in rats. Anesthesiology 103:360 –376.

Sarantopoulos CD, McCallum JB, Rigaud M, Fuchs A, Kwok W-M, Hogan QH (2007) Opposing effects of spinal nerve ligation on calcium-activated potassium currents in axotomized and adjacent mammalian primary afferent neurons. Brain Res 1132:84 –99.

Schulz DJ, Goaillard JM, Marder E (2006) Variable channel expression in identified single and electrically coupled neurons in different animals. Nat Neurosci 9:356 –362.

Schwab ME, Bartholdi D (1996) Degeneration and regeneration of axons in the lesioned spinal cord. Physiol Rev 76:319 –370.

Shaw AC, Holmes T, Johns JL, Thurman S, Jackson AW, Davis GR, McClellan AD (2001) Pathfinding of regenerating descending axons in spinal cord-transected larval lamprey: functional anatomy. Soc Neurosci Abstr 27:960.

Titmus MJ, Faber DS (1990) Axotomy induced alterations in the electrophysiological characteristics of neurons. Prog Neurobiol 35:1–51.

Tseng GF, Prince DA (1996) Structural and functional alterations in rat corticospinal neurons after axotomy. J Neurophysiol 75:248 –267.

Wallen P, Buchanan JT, Grillner S, Hill RH, Christenson J, Hokfelt T (1989) Effects of 5-hydroxytryptamine on the afterhyperpolarization, spike frequency regulation, and oscillatory membrane properties in lamprey spinal cord neurons. J Neurophysiol 61:759 –768.

Wikström MA, El Manira A (1998) Calcium influx through N- and P/Q-type channels activate apamin-sensitive calcium-dependent potassium channels generating the late afterhyperpolarization in lamprey spinal neurons. Eur J Neurosci 10:1528 –1532.

Yin HS, Wellerstein KK, Selzer ME (1981) Effects of axotomy on lamprey spinal neurons. Exp Neurol 73:750 –761.

Zhang L, McClellan AD (1999) Axonal regeneration of descending brain neurons in larval lamprey demonstrated by retrograde double labeling. J Comp Neurol 410:612– 626.

Zhang L, Palmer R, McClellan AD (2002) Increase in descending brain-spinal cord projections with age in larval lamprey: implications for spinal cord injury. J Comp Neurol 447:128 –137.