Experimental Neurology 300 (2018) 201–211

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Is more always better? How different 'doses' of exercise after incomplete spinal cord injury affects the membrane properties of deep dorsal horn interneurons

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ARTICLE INFO

Keywords:
Spinal cord injury
Patch clamp electrophysiology
Interneurons
Exercise
Intrinsic properties

ABSTRACT

Interneurons in the deep dorsal horn (DDH) of the spinal cord process somatosensory input, and form an important link between upper and lower motoneurons to subsequently shape motor output. Exercise training after SCI is known to improve functional motor recovery, but little is known about the mechanisms within spinal cord neurons that underlie these improvements. Here we investigate how the properties of DDH interneurons are affected by spinal cord injury (SCI) alone, and SCI in combination with different 'doses' of treadmill exercise training (3, 6, and 9 wks). In an adult mouse hemisection model of SCI we used whole-cell patch-clamp electrophysiology to record intrinsic, AP firing and gain modulation properties from DDH interneurons in a horizontal spinal cord slice preparation. We find that neurons within two segments of the injury, both ipsi- and contralateral to the hemisection, are similarly affected by SCI and SCI plus exercise. The passive intrinsic membrane properties input resistance (R_{in}) and rheobase are sensitive to the effects of recovery time and exercise training after SCI thus altering DDH interneuron excitability. Conversely, select active membrane properties are largely unaffected by either SCI or exercise training. SCI itself causes a mismatch in the expression of voltage-gated subthreshold currents and AP discharge firing type. Over time after SCI, and especially with exercise training (9 wks), this mismatched expression is exacerbated. Lastly, amplification properties (i.e. gain of frequency-current relationship) of DDH interneurons are altered by SCI alone and recover spontaneously with no clear effect of exercise training. These results suggest a larger 'dose' of exercise training (9 wks) has a strong and selective effect on specific membrane properties, and on the output of interneurons in the vicinity of a SCI. These electrophysiological data provide new insights into the plasticity of DDH interneurons and the mechanisms by which exercise therapy after SCI can improve recovery.

1. Introduction

Neurons in the spinal cord exhibit specific intrinsic and action potential (AP) discharge properties that arise from the combined activity of voltage-gated ion channels in their cell membranes (Morisset and Nagy, 1999; Reali et al., 2011; Ruscheweyh and Sandkuhler, 2002). In the dorsal horn of the spinal cord this complex inventory of membrane conductances plays a crucial role in determining the integrative capacity of neurons and their response to sensory input (Arshavsky, 2003; Grudt and Perl, 2002; Reali et al., 2011; Sandkuhler, 2009; Smith and Perrier, 2006). Importantly, these conductances can change after injury and pathology to alter the input-output function of dorsal horn neurons (Derjean et al., 2003; Dobremez et al., 2005; Lu et al., 2009; Reali et al., 2011).

Neurons located in the deep dorsal horn (DDH) of the spinal cord (laminae III–V) process somatosensory input and subsequently shape motor output (Morisset and Nagy, 1998; Tracey, 2004). In primates and rodents, descending inputs from axons of the corticospinal tract (CST) also terminate in the DDH (Galea and Darian-Smith, 1994; Tracey, 2004). This means DDH interneurons serve as the all-important 'link'

Abbreviations: AHP, afterhyperpolarization; AP, action potential; ACSF, artificial cerebrospinal fluid; CC, contralateral caudal; CE, contralateral epicentre; CR, contralateral rostral; CST, corticospinal tract; DC, dorsal column; DDH, deep dorsal horn; DF, delayed firing; ES, effect size; I_{Ar}, rapid I_A current; I_{As}, slow I_A current; IB, initial bursting; IC, ipsilateral caudal; I_{Ca}, T-type calcium current; i.p., intraperitoneal; IR, ipsilateral rostral; R_{in}, input resistance; RMP, resting membrane potential; sACSF, sucrose substituted artificial cerebrospinal fluid; s.c., subcutaneous; SCI, spinal cord injury; SS, single spiking; TF, tonic firing

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https://doi.org/10.1016/j.expneurol.2017.11.007
Received 16 August 2017; Received in revised form 22 October 2017; Accepted 12 November 2017
Available online 14 November 2017
0014-4886/

between upper and lower motoneurons. Thus, the intrinsic properties of DDH interneurons are critical for shaping responses to somatosensory input and for producing meaningful motor behaviors via their input onto ventral horn motoneurons (Cote and Gossard, 2004; Cote et al., 2003; Husch et al., 2012).

Following spinal cord injury (SCI) both descending motor and ascending somatosensory pathways are disrupted, resulting in severe motor and sensory defects. At the level of lower, or spinal, motoneurons we know that SCI can dramatically alter electrophysiological properties, particularly the way motoneurons fire action potentials (Barbeau and Rossignol, 1987; Beaumont and Gardiner, 2002; Lovely et al., 1990; Petruska et al., 2007; Thaweerattanasinp et al., 2016). Considerable data also suggest voltage-activated currents and intrinsic properties change in motoneurons after SCI (Harvey et al., 2006a, 2006b; Rank et al., 2007, 2011). Surprisingly, the effect of SCI on the intrinsic properties of other spinal cord neuron populations, such as the DDH interneurons, that are essential for processing sensory information and relaying motor commands from higher centres, is not well understood.

Exercise training after SCI is known to exert powerful effects on spinal cord plasticity, including anatomical changes such as enhanced sprouting of collaterals from axons near the SCI (Bareyre et al., 2004; Courtine et al., 2008; Engesser-Cesar et al., 2007; Fouad et al., 2001; Goldshmit et al., 2008). Although exercise-induced plasticity within spinal cord networks is accompanied by improvements in functional recovery (Battistuzzo et al., 2017; Behrman et al., 2006; de Leon et al., 1998; Edgerton et al., 2004), little is known about the mechanisms within neurons that underlie these exercise-mediated improvements. Recently we demonstrated that two shorter 'doses' of treadmill exercise training (3 and 6 wks) after a spinal cord hemisection did not markedly affect intrinsic membrane properties of spinal DDH interneurons but preferentially enhanced synaptic activity, both local and descending, in adult SCI mice The specific effects of longer durations of aerobic exercise training on the intrinsic membrane, AP discharge and underlying voltage-activated subthreshold currents of these DDH neurons is unknown. This is especially important since longer durations of treadmill exercise training (9 wks) are known to provide demonstrable improvements to locomotion in animal models of incomplete SCI (Battistuzzo et al., 2016).

Given the central role of intrinsic membrane properties in determining the overall function of neural networks, establishing the effects of exercise training on these properties is critical. This is particularly the case for DDH interneurons, as this interneuron population is essential for conveying locomotor drive from the cortex and shaping functional motor outputs via their input onto motoneurons (Cote and Gossard, 2004; Cote et al., 2003; Tracey, 2004). We therefore hypothesised that SCI would disrupt intrinsic membrane properties, but these properties would spontaneously 'recover' to control levels with time after SCI. We also predicted that longer durations of treadmill exercise training (i.e. 6 wks and 9 wks) after SCI would have a beneficial effect on intrinsic membrane properties, and return values to control levels sooner. Our analysis included an age-matched uninjured control group to control for changes in DDH neuron properties that accompany the normal ageing process.

2. Materials and methods

2.1. Ethical approval

The University of Newcastle Animal Care and Ethics Committee provided approval for all procedures as covered by the NSW Animal Research Act, 1985 (Approval Number A-2009-154).

This study builds on previous work where we examined the effects of SCI and small 'doses' (3 and 6 wks) of exercise training on the intrinsic and synaptic properties of interneurons in the vicinity of a SCI (Flynn et al., 2013; Rank et al., 2015a, 2015b). Our previous work was a broad survey of both intrinsic and synaptic properties of DDH neurons, and how these are changed by limited durations (3 and 6 wks) of exercise training (Flynn et al., 2013; Rank et al., 2015a) or by SCI alone (Rank et al., 2015b). Here we conduct a specific analysis of passive and active intrinsic properties of spinal cord DDH interneurons in SCI mice, with new comparisons following 3, 6 and 9 wks of aerobic exercise training. In this study we have also undertaken highly detailed quantitative analysis of firing characteristics of DDH interneurons. This provides new information on the summative output of interneurons in the vicinity of a SCI, including frequency-current relationships and amplification properties (gain modulation) that are determined by underlying intrinsic membrane properties. Importantly, we include measurements made from age-matched mice that did not receive SCI. We term these animals controls and have included them because neuron properties are known to change during postnatal development (Baccei and Fitzgerald, 2005; Tadros et al., 2012; Walsh et al., 2009). Thus, three experimental groups are included in our study: untrained and trained SCI mice and control mice. Mice were randomly allocated to trained or untrained groups using allocation concealment and investigators were blinded to experimental group.

2.2. Spinal cord injury surgery and exercise training procedures

The procedures for spinal cord hemisection surgery and exercise training have been described previously (Flynn et al., 2013; Rank et al., 2015a, 2015b), and are only briefly summarised here. Adult male C57/Bl6 mice (aged 9–10 wks) received a left T10 spinal cord hemisection (between T10 and T11 spinal nerves) under isoflurane anaesthesia. Buprenorphine (0.1 mg/kg s.c., every 8 h for 48 h) and carprofen (5 mg/kg s.c, every 24 h for 5 days) were administered for post surgical analgesia. The same person performed all surgeries. All SCI animals received 2 wks of treadmill training prior to their surgery to familiarise them with equipment and the aerobic exercise protocol.

After SCI surgery mice were allowed to recover for 1 wk and were randomly allocated to untrained or trained groups. Treadmill trained mice completed 2 × 10 min sessions of enforced treadmill exercise each day (5 days/wks) for a period of 3 (n = 12 mice), 6 (n = 14 mice) or 9 wks (n = 29 mice). Untrained mice remained in their cages, for passive recovery, during this time (3 wks n = 14 mice; 6 wks n = 13 mice; 9 wks n = 26). Treadmill speeds began at 6 m/min at 1 wk after surgery and progressed to 10–12 m/min over the training period. Mice in the control group were age-matched to SCI mice that completed 3 wks (~13 wks old; n = 19 mice), 6 wks (~16 wks old; n = 13 mice) or 9 wks (~19 wks old; n = 15) of treadmill training. Control mice were uninjured and received no treadmill training.

2.3. Tissue preparation

SCI mice were euthanized for in vitro whole-cell patch-clamp electrophysiology at the completion of their treadmill training or matched passive recovery times. Control mice were sacrificed at the appropriate age. Under deep ketamine anaesthesia (100 mg/kg i.p.) animals were decapitated and horizontal spinal cord slices were prepared as previously described in Flynn et al. (2013). Briefly, the mouse torso was submerged in ice-cold, oxygenated, sucrose substituted artificial cerebrospinal fluid (sACSF containing in mM: 250 sucrose, 25 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 6 $MgCl_2$, and 1 $CaCl_2$; pH 7.3) and a length of spinal cord (T5–T13) was isolated using a ventral approach. The spinal cord was then mounted on an agar cutting stage using cyanoacrylate glue. Horizontal slices (250 μm thick) were cut using a vibrating microtome (HM 650V; Microm, Walldorf, Germany or VT12 00s, Leica Microsystems, Nuslock, Germany). The slice containing the DDH and a continuous strip of the dorsal columns was selected (Fig. 1A) and transferred to a recording bath for whole-cell patch clamp electrophysiology. Slices were perfused with oxygenated ACSF containing (in mM): 118 NaCl, 25 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 1 $MgCl_2$, and 2.5 $CaCl_2$. The spinal cord slice was secured in the bath