Respiratory Physiology & Neurobiology 169 (2009) 94–101

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Respiratory recovery following high cervical hemisection☆

M.S. Sandhu[a], B.J. Dougherty[a,b], M.A. Lane[b], D.C. Bolser[c], P.A. Kirkwood[d], P.J. Reier[b], D.D. Fuller[a,*]

[a] Department of Physical Therapy, College of Public Health and Health Professions, McKnight Brain Institute, University of Florida, P.O. Box 100154, 100 S. Newell Drive, Gainesville, FL 32610, USA
[b] Department of Neuroscience, College of Medicine, McKnight Brain Institute, University of Florida, P.O. Box 100244, 100 S. Newell Drive, Gainesville, FL 32610-0244, USA
[c] Department of Physiological Sciences, College of Veterinary Medicine, P.O. Box 100144, 1600 SW Archer Rd, Gainesville, FL 32610-0144, USA
[d] Sobell Department for Motor Neuroscience and Movement Disorders, UCL, Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

ARTICLE INFO

Keywords:
Spinal cord injury
Breathing
Phrenic
Plasticity

ABSTRACT

In this paper we review respiratory recovery following C2 spinal cord hemisection (C2HS) and introduce evidence for ipsilateral (IL) and contralateral (CL) phrenic motor neuron (PhrMN) synchrony post-C2HS. Rats have rapid, shallow breathing after C2HS but ventilation ($\dot{V}_E$) is maintained. $\dot{V}_E$ deficits occur during hypercapnic challenge reflecting reduced tidal volume (VT), but modest recovery occurs by 12 wks post-injury. IL PhrMN activity recovers in a time-dependent manner after C2HS, and neuroanatomical evidence suggests that this may involve both mono- and polysynaptic pathways. Accordingly, we used cross-correlation to examine IL and CL PhrMN synchrony after C2HS. Uninjured rats showed correlogram peaks consistent with synchronous activity and common synaptic input. Correlogram peaks were absent at 2 wks post-C2HS, but by 12 wks 50% of rats showed peaks occurring with a 1.1 ± 0.19 ms lag from zero on the abscissa. These data are consistent with prolonged conduction time to IL (vs. CL) PhrMNs and the possibility of polysynaptic inputs to IL PhrMNs after chronic C2HS.

1. Introduction

Hemisection from the midline to lateral edge of the cervical spinal cord has been used extensively to study respiratory plasticity following spinal cord injury (SCI) (Goshgarian, 2003; Fuller et al., 2005b; Lane et al., 2008a). The basic premise is that C2 hemisection (C2HS) interrupts descending bulbospinal pathways from the medulla to phrenic motoneurons (PhrMNs) located ipsilateral (IL) to the lesion. Thus, the IL hemidiaphragm is transiently paralyzed but contralateral (CL) diaphragm activity persists. Compensation via activation of CL PhrMNs and other respiratory pathways is sufficient to maintain minute ventilation ($\dot{V}_E$) thereby enabling the animal to survive the lesion. Subsequently, recovery processes affecting respiratory motor output can be studied. The purpose of this article is two-fold. First, we will review and summarize current knowledge regarding respiratory recovery and compensation following C2HS. Second, we will present original neurophysiological data from our laboratory which provides insight into the potential neural substrate for recovery of IL phrenic activity (i.e. the crossed phrenic phenomenon or CPP) following chronic C2HS.

2. The C2HS model and midline tissue sparing

Prior to discussing the functional impact of C2HS on breathing, a brief discussion regarding the "anatomical completeness" of cervical hemilesion injury is warranted. Confusion arises regarding the following question: for demonstration of the CPP, does a hemilesion need to extend to the spinal midline, or can some ventromedially (VM) located white matter be spared? Perhaps the most critical observation is that a portion of the bulbospinal axons innervating IL PhrMNs are found in the VM cervical white matter (Lipski et al., 1994; Fuller et al., 2009). Accordingly, one might predict that cervical hemilesions which spare the VM spinal cord will also fail to abolish inspiratory bursting in IL PhrMNs. This prediction is supported by the data of Li et al. (2003), but others have reported that partial cervical hemilesions abolish (at least transiently) IL phrenic output (Goshgarian, 1981; Vinit et al., 2007). We recently compared phrenic motor output and $\dot{V}_E$ between C2 hemilesioned rats with a small degree of VM white matter sparing vs. rats with anatomically complete C2HS (Fuller et al., 2009) (see Fig. 1). The group with spared VM pathways showed greater tidal volume (VT) and more robust IL phrenic bursting during chemical respiratory challenge. Moreover, the incompletely lesioned rats had lower breathing frequency (fR) during both unanesthetized and anesthetized conditions. Accordingly, we suggest that medially located fibers can impact respiratory outcomes after cervical hemilesion (Fuller et al., 2009). Although lateral hemilesion with

☆ This paper is part of a special issue entitled 'Spinal cord injury—Neuroplasticity and recovery of respiratory function', guest-edited by Gary C. Sieck and Carlos B. Mantilla.

* Corresponding author. Tel.: +1 352 273 6634; fax: +1 352 273 6109.
E-mail address: ddf@phhp.ufl.edu (D.D. Fuller).

1569-9048/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.resp.2009.06.014

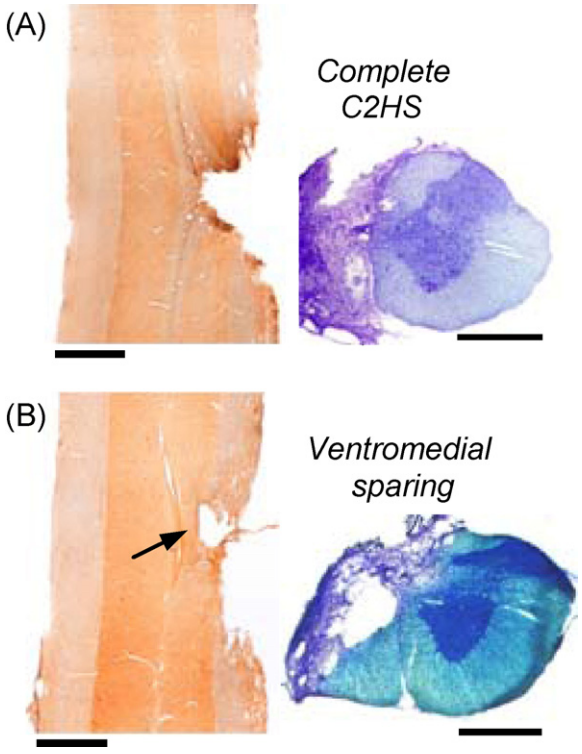

Fig. 1. Representative histological sections of the cervical spinal cord following hemilesion. Panel A presents both longitudinal and transverse sections of a anatomically complete C2HS injury. Panel B provides longitudinal and transverse sections of an incomplete C2HS with ventromedial tissue sparing. The arrow in panel B indicates the area of midline tissue sparing. The longitudinal sections (40 μm, vibratome) are labeled with antibodies to pseudo-rabies virus (PRV; as part of a separate study); the transverse sections (40 μm, vibratome) are stained with luxol fast blue and cresyl violet. Scale bars are 1 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

VM tissue sparing is an important model with some key experimental advantages (Kastner and Gauthier, 2008), in this review we will emphasize studies with anatomically or physiologically confirmed C2HS lesions.

3. Recovery of respiratory motor function after C2HS

3.1. Recovery, plasticity and compensation

Cervical SCI always alters breathing, and both humans as well as experimental animals often show some degree of respiratory recovery following chronic cervical SCI (Nantwi et al., 1999b; Winslow and Rozovsky, 2003; Fuller et al., 2008). However, respiratory recovery in mice may be blunted relative to other species (see Seeds et al., this volume). For this report we define recovery as the return of ($\dot{V}_E$) or other indices of respiratory function (e.g. vital capacity, nerve activity, etc.) towards values that would be expected in spinal-intact individuals of comparable age, sex and body mass. The recovery process can occur through a range of mechanisms, many of which represent types of *neuroplasticity*. A working definition was put forth by Mitchell and Johnson (2003) who defined respiratory-related neuroplasticity as "a persistent change in the neural control system (morphology and/or function) based on prior experience". Within the context of this definition, it is useful to differentiate between *compensation* (or compensatory plasticity) in neurologically intact pathways vs. *plasticity* in neurons and/or networks directly impaired by C2HS (e.g. denervated IL PhrMNs; see also Lane et al. this volume). While both these processes fit under the broad definition of plasticity (i.e. a "persistent change", Mitchell and Johnson, 2003), the underlying mechanisms are likely much different. Compensation after SCI, for example, involves increased recruitment of other respiratory muscles via intact neural pathways, and may reflect persistently altered sensory feedback (Teitelbaum et al., 1993; Katagiri et al., 1994; Brichant and De Troyer, 1997). On the other hand, changes in synaptic connections or other adaptations that increase the efficacy of neurotransmission to IL PhrMNs are examples of plasticity in a neurologically impaired pathway. Thus, the terms plasticity and compensation, while not mutually exclusive, generally are used to describe mechanistically different processes. In this manuscript we will use the term compensation to describe alterations in CL respiratory output and/or accessory muscle recruitment after C2HS.

3.2. Ventilation after C2HS

If the C2HS model is to be used to test potential therapeutic interventions (e.g. theophyline (Nantwi et al., 1996), cAMP (Kajana and Goshgarian, 2008), hypoxia (Golder and Mitchell, 2005), light-activated proteins (Alilain et al., 2008)), then it is important to determine how this injury impacts $\dot{V}_E$ and related behaviors such as augmented breaths. An important caveat is that while $\dot{V}_E$ measures can describe the recovery of breathing, such measures will reveal little about the mechanism of recovery (e.g. plasticity and compensation). Nevertheless, behavioral measures such as $\dot{V}_E$ provide an important complement to neuroanatomical, neurophysiological, and molecular studies of respiratory plasticity after SCI.

To date, several studies have examined the impact of C2HS on $\dot{V}_E$ (Goshgarian et al., 1986; Nantwi et al., 1999a; Golder et al., 2001b, 2003; Fuller et al., 2006, 2008). The initial report was from Goshgarian et al. (1986) who demonstrated that female rats breathe with an elevated frequency at approximately 24 h post-C2HS. Arterial blood gases were consistent with hyperventilation as reflected by increased arterial pO_2 and a tendency for decreased arterial pCO_2. It was subsequently reported that rats transiently hypoventilate for a few hours after C2HS (Fuller et al., 2005b) but by 2-wks post-injury their arterial blood gases are not different than uninjured control rats (Miyata et al., 1995). Golder et al. (2001b) used pneumotachography to study the pattern of breathing in anesthetized, tracheotomized C2HS female rats breathing room air. Relative to uninjured control animals, C2HS rats had increased breathing frequency (fB) and reduced VT at both 1 and 2 mos post-injury. Further, bilateral vagotomy caused the rapid, shallow breathing pattern to return to control values thereby demonstrating that vagal mechanisms contribute to the pattern of breathing after C2HS. Golder et al. (2001b) also showed that sighs or augmented breaths occur more frequently after C2HS. Augmented breaths prevent lung atelectasis and are impaired in human SCI (McKinley et al., 1969). Thus, the augmented breath is a potentially useful outcome measure in pre-clinical rodent SCI models (see Bolser et al., this volume).

The first investigation of ventilation in unanesthetized, unrestrained rats after chronic C2HS was conducted by Fuller et al. (2006) using barometric plethysmography (see Mortola and Frappell (1998) for review and commentary on the method). In that study, $\dot{V}_E$ was examined in male rats during a baseline period (21% O_2) and a hypercapnic challenge (21%O_2, 7% CO_2) at 2–5 wks post-injury. The respiratory challenge is particularly important because chemical stimulation of breathing (i.e. hypoxia, hypercapnia) can activate IL phrenic pathways after C2HS (see below). Rats maintained $\dot{V}_E$ with a rapid, shallow breathing pattern (reduced VT, increased fB) that persisted through the duration of the study. Deficits in $\dot{V}_E$ were revealed during the hypercapnic challenge and reflected reduced VT. There was no evidence for recovery of VT or $\dot{V}_E$ over the 5-wk-post-injury period. However, in that study VT or $\dot{V}_E$ were compared to pre-injury measurements in the same rats