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Short Communication

Supraspinal respiratory plasticity following acute cervical spinal cord injury

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ABSTRACT

Impaired breathing is a devastating result of high cervical spinal cord injuries (SCI) due to partial or full denervation of phrenic motoneurons, which innervate the diaphragm – a primary muscle of respiration. Consequently, people with cervical level injuries often become dependent on assisted ventilation and are susceptible to secondary complications. However, there is mounting evidence for limited spontaneous recovery of respiratory function following injury, demonstrating the neuroplastic potential of respiratory networks. Although many studies have shown such plasticity at the level of the spinal cord, much less is known about the changes occurring at supraspinal levels post-SCI. The goal of this study was to determine functional reorganization of respiratory neurons in the medulla acutely (>4 h) following high cervical SCI. Experiments were conducted in decerebrate, unanesthetized, vagus intact and artificially ventilated rats. In this preparation, spontaneous recovery of ipsilateral phrenic nerve activity was observed within 4 to 6 h following an incomplete, C2 hemisection (C2Hx). Electrophysiological mapping of the ventrolateral medulla showed a reorganization of inspiratory and expiratory sites ipsilateral to injury. These changes included i) decreased respiratory activity within the caudal ventral respiratory group (cVRG; location of bulbospinal expiratory neurons); ii) increased proportion of expiratory phase activity within the rostral ventral respiratory group (rVRG; location of inspiratory bulbo-spinal neurons); iii) increased respiratory activity within ventral reticular nuclei, including lateral reticular (LRN) and paragigantocellular (LPGi) nuclei. We conclude that disruption of descending and ascending connections between the medulla and spinal cord leads to immediate functional reorganization within the supraspinal respiratory network, including neurons within the ventral respiratory column and adjacent reticular nuclei.

1. Introduction

Injury at nearly any spinal level can affect breathing due to the wide distribution of respiratory lower motoneurons from cervical to lumbar spinal segments (see review (Lane, 2011)). However, the most devastating effects on respiratory function arise following spinal cord injury (SCI) at high- to mid-cervical spinal levels. Among the vast range of motoneurons that become denervated following such injuries are the phrenic motoneurons (distributed from C3 to C5/6 in rats) (Goshgarian and Rafols, 1984; Kuzuhara and Chou, 1980; Lane, 2011; Lane et al., 2008; Mantilla et al., 2009), which innervate the diaphragm – the primary muscle of respiration. In the intact spinal cord, phrenic motoneurons receive bilateral input from neurons in the ventral respiratory column (VRC) as depicted in the schematic diagram in Fig. 1. These projections run through lateral and ventromedial funiculi (Fuller et al., 2009; Lipski et al., 1994), and synapse onto motoneurons directly or indirectly via pre-phrenic interneurons (Lane et al., 2008).

Compromised phrenic circuitry and subsequent diaphragm paralysis typically lead to respiratory arrest and the need for assisted ventilation (Baydur and Sassoon, 2010; Como et al., 2005; Wong et al., 2012). There is mounting clinical and experimental evidence, however, for some degree of spontaneous improvement in respiratory function post-injury, reflecting the neuroplastic potential within the respiratory network. Although previous research in this field has been focused on mechanisms of plasticity within the spinal cord (Goshgarian, 2009; Lane et al., 2009; Mitchell and Johnson, 2003; Warren et al., 2013), neuroplastic changes may appear at multiple levels post-SCI including the periphery, spinal cord, brainstem, and brain. One example is the somatotopic reorganization of primary somatosensory and motor cortices after SCI as reported in many experimental and clinical studies (Endo et al., 2007; Humanes-Valera et al., 2013; Kokotilo et al., 2009; Lotze et al., 1999). Not surprisingly, neuroplastic changes in the brainstem post-SCI affecting motor control have gained increasing attention (Kumru et al., 2009; Weishaupt et al., 2013; Zorner et al., 2014). While some previous work has examined potential neuroplastic mechanisms within brainstem

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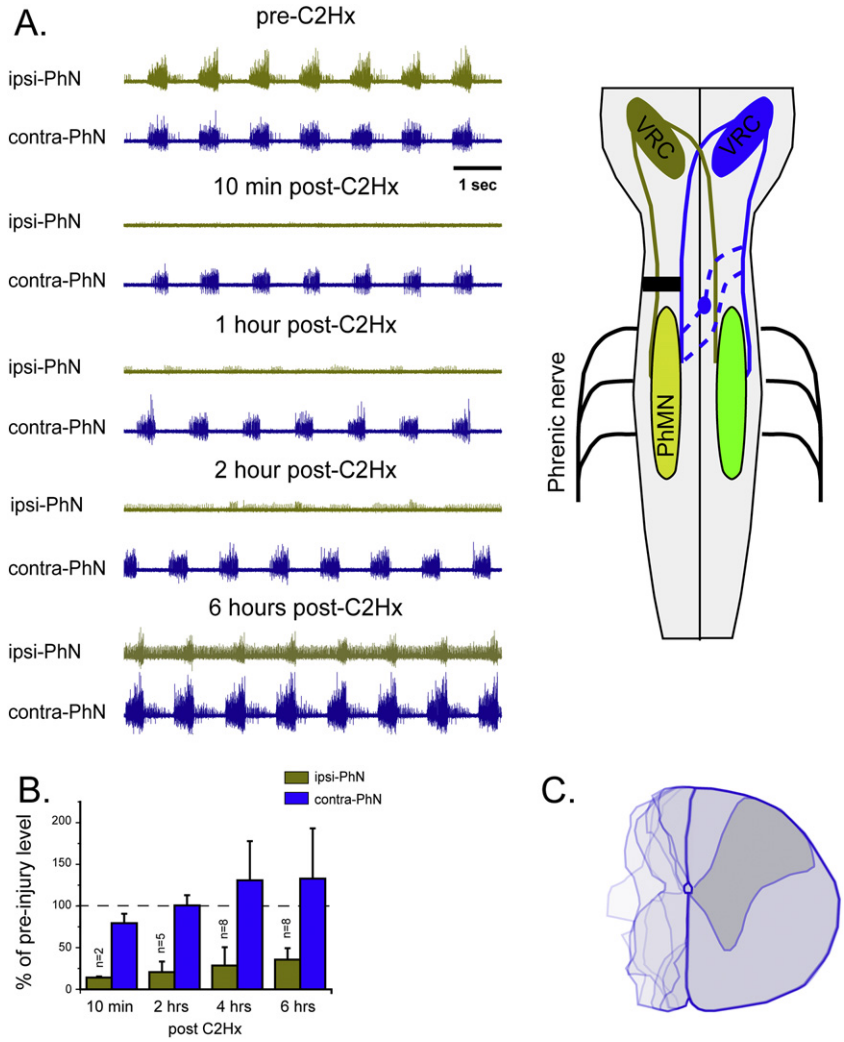

Fig. 1. Recovery of phrenic activity post-C2Hx. A. The schematic diagram illustrates connectivity between ventral respiratory group (VRC) and spinal phrenic motoneurons following C2Hx. Blue dot represents phrenic interneurons. Phrenic motor output was determined by electrophysiological recording from the phrenic nerves (PhN): ipsi-PhN, contra-PhN before, 10 min, 1, 2 and 6 h post C2Hx. B. Ipsilateral and contralateral phrenic nerve recovery as a percentage of pre-injury level at 10 min, 2, 4 and 6 h post C2Hx. C. Reconstruction of cross-sections through the lesion epicenter shows that not all injuries were complete hemisections.

respiratory networks (Morris et al., 2003; Morris et al., 2000; Morris et al., 2001), few studies have focused on supraspinal plasticity following SCI (Buttry and Goshgarian, 2014; Felix et al., 2014; Golder et al., 2001; Vinit et al., 2005). For instance, previous work has found that cells within the VRC and raphe nuclei ipsilateral to a partial C2 hemisection (C2Hx) show increased Akt signaling which decreases cytoplasmic phosphorylated forkhead transcription factor (P-FKHR) immunoreactivity (Felix et al., 2014). Furthermore, phrenic motor recovery appears to be at least in part dependent on FKHR phosphorylation within brainstem respiratory nuclei (Felix et al., 2014). Studies in the neonate also revealed evidence for brainstem plasticity following C2Hx with increases in glutamatergic and adenosinergic receptors within the ipsi- and contralateral medulla (Zimmer and Goshgarian, 2007). A recent study by Buttry and Goshgarian (2014) using transynaptic WGA-Alexa 488 to trace the phrenic motor circuit shows an increased number of structures with labeled neurons in the brainstem following chronic C2Hx injury in rats. These structures include: parvicellular reticular, gigantocellular reticular, and intermediate reticular nuclei. The authors concluded that plasticity occurs at the supraspinal level to re-establish function on the injured side by the activation of normally latent compensatory pathways, and that these pathways may be mediated by reticular nuclei (Buttry and Goshgarian, 2014). Collectively, these results show that brainstem neurons not only remain connected to the spinal cord post-injury, but they represent an unexplored therapeutic target.

Effective therapeutic targeteting of brainstem neurons, however, may require a better udnerstanding of how fucntional states are altered post-injury. The discharge pattern of individual brainstem neurons in pre-injury and post-injury states are still unknown. The present work is the first to examine how acute C2Hx impacts patterns of inspiratory or expiratory activity within bulbospinal neurons of the VRC and adjacent reticular nuclei. We hypothesized that interruption of bulbospinal and spinobulbar pathways directly affects the activity of ipsilateral bulbospinal and surrounding respiratory neurons within hours. The present data support this hypothesis and provide the first experimental evidence for i) reorganization of inspiratory and expiratory activity within the VRC, and ii) increased respiratory activity in the ventral reticular nuclei acutely (within hours) following cervical SCI.

2. Materials and methods

Experiments were conducted on male Sprague-Dawley rats (400–450 g; Harlan Laboratories, Inc.). Animals were divided into two groups: uninjured controls ($n = 9$) and incomplete (see 'Results' and Fig. 1C for details) C2Hx ($n = 8$). All surgical and animal care procedures were approved by the Drexel University Institutional Animal Care and Use