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Treatments to restore respiratory function after spinal cord injury and their implications for regeneration, plasticity and adaptation

Himanshu Sharma, B.S., Warren J. Alilain, Ph.D., Anita Sadhu, B.S., and Jerry Silver, Ph.D.
Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, USA

Abstract

Spinal cord injury (SCI) often leads to impaired breathing. In most cases, such severe respiratory complications lead to morbidity and death. However, in the last few years there has been extensive work examining ways to restore this vital function after experimental spinal cord injury. In addition to finding strategies to rescue breathing activity, many of these experiments have also yielded a great deal of information about the innate plasticity and capacity for adaptation in the respiratory system and its associated circuitry in the spinal cord. This review article will highlight experimental SCI resulting in compromised breathing, the various methods of restoring function after such injury, and some recent findings from our own laboratory. Additionally, it will discuss findings about motor and CNS respiratory plasticity and adaptation with potential clinical and translational implications.

Spinal cord injuries often occur at the cervical level. For the injured patient, this is detrimental because of the immediate threat to the important function of breathing. The final common pathway motor neurons from the ventral horn to the two halves of the diaphragm, the phrenic nuclei (PN), are located bilaterally at the C3 to C6 level of the spinal cord. Therefore, cervical SC trauma can potentially disrupt axons from the respiratory regions of the brainstem to the PN or damage the phrenic motor neurons themselves - resulting in paralysis of the diaphragm and impaired breathing (Fuller et al, 2009, Goshgarian, 2003, Moreno, et al., 1992). In addition, compromised respiration makes the SCI patient more susceptible to respiratory related illness and disease, including pneumonia and atelectasis. Clearly, investigating interventions to restore breathing and understanding the adaptive changes, which take place after SCI are of high priority to the SCI community.

The majority of studies exploring the ramifications of SCI on breathing and potential therapies utilize the lateral C2 hemisection model (Fuller, et al., 2009, Goshgarian, 2003, Zimmer, et al., 2007). With this injury type, projections from the rostral ventral respiratory group (rVRG) in the medulla, which provide the excitatory inspiratory drive to the ipsilateral PN, are disrupted (Fig. 1) (Goshgarian and Rafols, 1981, Goshgarian and Rafols, 1984, Moreno, et al., 1992, Chitravanshi and Sapru, 1996). As a result, the hemidiaphragm

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Corresponding Author: Jerry Silver, Ph.D., Department of Neurosciences, Case Western Reserve University School of Medicine, 2109 Adelbert Road, Cleveland, OH 44106, jxs10@case.edu.

Please note that Himanshu and Warren are co-first authors on this article

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ipsilateral to the lesion becomes paralyzed. The injured animal is able to breathe independent of a mechanical ventilator since the hemidiaphragm contralateral to the hemisection is still active and compensates for the decrement in respiratory capacity. From here, strategies to restore function can be examined and the plasticity and adaptation which takes place after SCI associated with hemiparalysis of a major muscle can be uncovered.

It was demonstrated by Porter in 1895 that following C2 hemisection, activity could be restored to the paralyzed hemidiaphragm via activation of a spared, but diminutive and latent pathway to the denervated phrenic nucleus. Specifically, in these early experiments, it was found that when the contralateral phrenic nerve of a C2 hemisected animal was transected, instead of killing the animal due to complete paralysis of the diaphragm, the initially paralyzed hemidiaphragm became rapidly active again, allowing the animal to survive (Goshgarian, 2003, Goshgarian, 1981, Rosenbluth and Ortiz 1936, Porter, 1895). This recovery following hemisection and contralateral phrenicotomy was called the “crossed phrenic phenomenon” (Goshgarian, 2003). The pathway mediating the recovery (the crossed phrenic pathway) circumvents the hemisection by descending contralateral to the lesion and crossing over at the level of the PN (Goshgarian, et al., 1991, Moreno, et al., 1992). Although the pathway continues to be a potential path for descending input following C2 hemisection alone, this pathway is normally latent and ineffective, since after hemisection, there is no function in the hemidiaphragm.

It was later demonstrated that activation of this pathway is dependent upon strongly increasing respiratory drive (Lewis and Brookhart, 1951). Since this discovery, there have been numerous studies to increase respiratory drive and activate this pathway without a contralateral phrenicotomy by utilizing pharmacological treatments, intermittent hypoxia, and reducing plasticity inhibiting substrates. In addition, this model presents an opportunity to examine methods to increase the effectiveness of spared pathways potentially remaining after any type of SCI to improve function or behavior.

Strategies to restore respiratory function after cervical SCI

Since it had been shown that increasing central respiratory drive via contralateral phrenicotomy after C2 hemisection reveals and activates this latent pathway, it was logical to explore whether pharmacological treatments that increase respiratory drive would also work in the C2 hemisected animal to restore function. Indeed, it was shown that administration of the methylxanthine, theophylline; a respiratory stimulant long used as a treatment for asthma, could partially restore function to the paralyzed hemidiaphragm. Through a series of experiments it was determined that the mechanism behind the restoration of activity was through theophylline’s non-specific antagonism of adenosine (and specifically adenosine A1) receptors (Nantwi, et al., 1996, Nantwi and Goshgarian, 1998). Focusing on the adenosine system, it appears that manipulation of both central and peripheral receptors can restore as well as modulate respiratory activity (Nantwi, 2009, Nantwi and Goshgarian, 2005). Additionally, chronic treatment with theophylline results in restored activity that persists even after weaning off the drug, suggesting that plasticity of the spinal respiratory circuitry could be induced via long term drug mediated recovery (Nantwi, et al., 2003). Additionally, the inhibitory effect of theophylline on phosphodiesterase, the enzyme which degrades cAMP, has also led to further experiments showing that upregulation of cAMP can lead to plasticity of the spinal respiratory circuitry and restoration of function (Kajana and Goshgarian, 2008, Kajana and Goshgarian, 2008).

As the theophylline experiments showed, pharmacologically increasing respiratory drive can result in restored hemidiaphragmatic activity following C2 hemisection. However, drug intervention is not the only way to increase CNS respiratory drive.

Intermittent hypoxia and plasticity of the intact respiratory system

Exposure to repeated episodes of hypoxia (i.e. low oxygen levels) interspersed with a return to normal oxygen levels has been shown to elicit a long-lasting increase in respiratory motor output or drive, a phenomenon known as long term facilitation (LTF) (for a review, see Fuller et al, 2000). LTF is a form of respiratory plasticity, generally studied in phrenic and hypoglossal nerves, that lasts at least one hour (although this threshold varies) and is primarily expressed as an enhanced nerve burst amplitude leading to increased inspiratory motor output (Bocchiario and Feldman 2004, Fuller et al 2001a). LTF patterns in the phrenic nucleus are known as phrenic LTF (pLTF), and are the focus of this section, although LTF in the hypoglossal nerve and ventilatory LTF (vLTF) will also be discussed.

LTF was originally discovered as a phenomenon arising from periodic stimulation of the carotid sinus nerve that persisted despite normal blood arterial gas levels (Millhorn et al, 1980a). This pattern of plasticity has also been shown to be elicited by intermittent hypoxia (Hayashi et al, 1993). Mostly developed and elucidated in the seminal studies from the Gordon Mitchell laboratory, it has been shown an array of protocols that present hypoxia to animals have been shown to elicit respiratory plasticity, making this a technique of interest. Understanding and elucidating the mechanisms of this phenomena may lead to understanding and even harnessing this latent potential for plasticity in the spinal cord, especially after injury. Different protocols induce contrasting effects, but the mechanisms seem to share common pathways.

The most extensively studied paradigm to induce phrenic LTF is acute intermittent hypoxia (AIH) which is characterized by 3–5 episodes of hypoxia each lasting for 5 minutes (Vinit, 2009; Hayashi et al, 1993). Less well studied is chronic intermittent hypoxia (CIH), which differs from AIH in that it may have over 500 such episodes over a period of days or weeks (Vinit, 2009). CIH pretreatment can enhance the effects of an AIH protocol by increasing sensitivity to hypoxia as well as increasing the phrenic response during hypoxia (Ling et al, 2001). It also increases the duration of LTF in AIH exposed rats but without changing baseline ventilation or metabolism (McGuire et al, 2003). These effects indicate that CIH induces metaplasticity (i.e., previous plastic changes which alter the current plasticity of circuits). This metaplasticity renders hypoxia more effective in inducing LTF when preceded with CIH (McGuire et al, 2003). Unfortunately, CIH protocols have several pernicious side effects including high blood pressure (Fletcher et al., 1992), cognitive deficits (Row et al, 2007; Row, 2007), as well as the so-called metabolic syndrome (Tasali and Ip, 2008).

Several other experimental protocols exist including daily acute intermittent hypoxia (dAIH) (Wilkerson and Mitchell, 2009) and early postnatal CIH (Reeves and Gozal, 2006). Of course, each protocol elicits different results and dAIH may especially allow researchers to circumvent the problems of CIH side effects (some dAIH results are discussed below). A final experimental protocol of note is chronic or sustained hypoxia exposure, which does not induce synaptic plasticity in the phrenic nucleus (Castro-Moure and Goshgarian, 1997).

It has been known since the discovery of LTF that the mechanism is intimately involved with the serotonergic system (Millhorn et al 1980b). Two years after Castro-Moure and Goshgarian's results, Kinkead and Mitchell discovered that 5-HT_{2A/C} receptor activation is necessary for the short-term phrenic response, but post-hypoxia frequency decline (which would be absent in chronic hypoxia) is also necessary for LTF (Kinkead and Mitchell, 1999). It also was demonstrated that intermittent serotonin activation of 5-HT receptors appears to trigger protein synthesis required for LTF (Baker-Herman and Mitchell, 2002). Further, it has been shown that periodic intraspinal injections of 5-HT receptor agonists

directly into the spinal cord can induce phrenic motor facilitation (MacFarlane and Mitchell, 2009). While serotonin is necessary for initiating phrenic LTF, it is not necessary to maintain it (Fuller et al, 2001a).

Blockade of NMDA receptors via pretreatment with the antagonist MK-801 in the phrenic motor nucleus has been shown to eliminate pLTF in anesthetized rats (McGuire et al, 2005). It has also been demonstrated that (2R)-2-amino-5-phosphonovaleric acid (APV)(an NMDA antagonist) can prevent AIH-induced vLTF and attenuate or abolish vLTF in conscious rats if administered at the time of AIH or even 20 minutes after AIH (McGuire et al, 2008). Unlike serotonin, NMDA receptor activation seems necessary to maintain LTF patterns. Nonetheless, both play major roles in mediating recovery after injury.

Overall, the pathway for pLTF seems to start when hypoxic episodes activate serotonergic medullary raphe neurons (Erickson and Millhorn, 1994), which in turn trigger serotonin release near phrenic motor neurons in the spinal cord. This release activates 5-HT₂ receptors, which are coupled to G_q proteins (for a review, see Bockaert et al. 2006). These G proteins (through the Phospholipase C pathway leading to activation of PKC) stimulate new protein synthesis and trigger a release of BDNF. The ensuing activation of BDNF's high affinity receptor TrkB has been shown to be necessary and sufficient for pLTF (Baker-Herman et al 2004). Changes occurring downstream of TrkB activation are less well understood, but it seems that extracellular regulated kinases 1 and 2 (ERK1/2) play important roles. BDNF increases their phosphorylation in motor neurons (Kishino and Nakayama, 2003) and AIH does the same in areas associated with the phrenic motor nucleus (Wilkerson and Mitchell, 2009). Further, when spinal MEK (the enzyme responsible for phosphorylating ERKs) is inhibited, pLTF is abolished (Dale-Nagle et. al, 2010). BDNF has been shown to modulate NMDA receptors by phosphorylating its subunit 1 (NR1) via this ERK pathway (Slack et al., 2004); possibly being the mechanism by which modulation of NMDA receptors induces pLTF patterns in phrenic motor neurons.

The reason that hypoxia must be intermittent is that sustained hypoxia induces certain serine/threonine phosphatases that prevent pLTF from developing. Inhibition of those phosphatases allows pLTF to develop during sustained hypoxia but has no effect on the patterns evoked by AIH (Wilkerson et al, 2008). This phosphatase activity can be blocked by the formation of reactive oxygen species (ROS), which is what seems to happen in AIH when 5-HT activated protein kinase C induces NADPH oxidase activity to increase ROS levels (MacFarlane et al, 2009; Mahamed and Mitchell, 2007). It appears the reason ROS levels are higher during intermittent hypoxia (vs. sustained hypoxia) is due to NADPH oxidase exhibiting a burst of ROS formation during re-oxygenation after oxidative stress (Abramov et al, 2007). Furthermore, inhibitors of the NADPH oxidase complex have been shown to attenuate pLTF indicating a crucial regulatory role of this complex in IH-induced LTF (MacFarlane et al, 2009).

In CIH, plasticity of the carotid bodies (the group of cells that play a chemosensory role and detect arterial blood gas levels) is also serotonin dependent, but appears to utilize a broader range of 5-HT receptors including the 5-HT₇ receptor (McGuire et al, 2004). CIH can cause LTF of carotid body afferent nerve activity (i.e., sensory LTF (sLTF) (Peng et al, 2003). sLTF requires conditioning with CIH, is reversible after exposure to normoxia, preventable with anti-oxidants (Peng et al, 2003) and like AIH, requires NADPH oxidase (Peng et al, 2009) suggesting similarities between the sLTF and pLTF pathways.

There are several other mechanisms and pathways by which phrenic motor output can be enhanced as well involving other pathways (Dale-Nagle et al, 2010), but these mechanisms do not appear to be central to hypoxia induced LTF.

Intermittent hypoxia and SCI

In the context of cervical SCI, intermittent hypoxia improves respiratory output. This improvement has been demonstrated by a protocol of AIH applied 4 to 8 weeks after C2 hemisection resulting in a serotonin-dependent plasticity (Golder and Mitchell, 2005), implying that AIH improves functional recovery even after chronic spinal cord injury. Further downstream in the putative pathway, Golder et al discovered that mimicking the impact of BDNF on TrkB receptors by activating adenosine 2A receptors also improved respiratory function after C2H. (Golder et al, 2008). Overall, this work points to a possibility of harnessing the AIH-induced serotonin dependent plasticity pathway to improve respiratory function after spinal cord injury.

CIH has also been demonstrated to improve phrenic motor output after C2 hemisection. Specifically, CIH given for 7 days after C2 hemisection improved spontaneous ipsilateral phrenic motor output via the crossed phrenic pathway in sub-chronically injured (2 weeks) rats. However, CIH preconditioning had no effect, and CIH also had no effect on contralateral outputs or in uninjured control rats (Fuller et al, 2003). Further, short latency phrenic potentials are stronger after CIH which points to increased efficiency of synapses in a single or pauci-synaptic pathway (Fuller et al, 2003). The CIH mediated plasticity has also been demonstrated to be serotonin-dependent (McGuire et al 2004). Interestingly, cervical spinal cord injury has been shown to upregulate spinal 5-HT_{2A} receptors in ventral segments ipsilateral to C2H associated with the phrenic motor nucleus (Fuller et al 2005). These results collectively suggest that CIH takes advantage of and strengthens crossed phrenic pathway circuitry that develops after chronic SCI through a serotonin-dependent mechanism. The upregulation of 5-HT_{2A} receptors may allow for greater plasticity after SCI than is available normally and improve synaptic efficiency in spared pathways.

Daily treatments of AIH (dAIH), which can avoid the negative side effects of CIH (Wilkerson and Mitchell, 2009), have also shown some exciting results in inducing plasticity and restoring ventilatory output. In C2H rats, dAIH returned tidal volume to the same level as sham-operated rats, although it did not entirely restore normal breathing patterns (Barr et al 2007).

Importantly, there is some evidence that intermittent hypoxia may improve non-respiratory motor function after spinal cord injury as well. Preliminary evidence taken from studies done on two SCI incomplete patients showed an increase in voluntary motor function in the ankle after treatment with 15 episodes of hypoxia. (Rymer et al, 2007, Trumbower et al, 2011).

Gaining a further understanding of the mechanisms by which intermittent hypoxia induces plasticity in the spinal cord offers a very intriguing and exciting avenue of research to help devise strategies to maximize the intrinsic capacity for spinal cord plasticity after injury. Furthermore, it seems that there is even greater potential for IH induced plasticity to improve both respiratory and non-respiratory related synaptic circuitry if sprouting or bona fide regeneration of damaged bulbospinal respiratory pathways can be achieved.

Sprouting and regeneration of respiratory CNS circuitry

Following SCI, growth of severed axons is very limited without intervention. In the face of the injury-induced environment of the “glial scar”, which is composed of potentially inhibitory reactive astrocytes and extracellular matrix molecules, transected axons terminate in dystrophic endbulbs unable to reach their proper neuronal targets (Busch and Silver, 2007, Cajal, 1928, Silver and Miller, 2004). This, however, does not mean that CNS axons do not have the intrinsic ability to grow and regenerate. When introduced to an environment

favorable to regeneration, or a “growth permissive” environment, injured axons can indeed grow. In their classic work, David and Aguayo (1981) showed that a segment of the peripheral nervous system (PNS) is one such substrate that can support and re-myelinate the lengthy regrowth of a large number of central axons (David and Aguayo, 1981). In the context of SCI, grafts of PNS segments can be used to bridge around a lesion and provide a new and growth-permissive route to denervated neurons. Re-entry of axons from the PNS graft back into a CNS environment, however, remains an issue in this approach.

There have been several attempts to bridge a lesion with peripheral nerve grafts to the phrenic motor nucleus in order to restore respiratory function. In their pioneering work, Gauthier and colleagues found that respiratory related bulbospinal axons can readily regenerate into a peripheral nerve bridge placed near the rVRG or into the funiculi that contain the descending respiratory tracts (Decherchi, et al., 1996, Gauthier and Lammari-Barreault, 1992, Gauthier and Rasminsky, 1988, Lammari-Barreault, et al., 1991). However, they also showed that a paucity of fiber penetration back into the CNS produced only minimal impact on restoring spontaneous phrenic nerve activity after a C3 hemi-lesion (Gauthier, et al., 2002). The aforementioned inhibitory ECM molecules could potentially contribute to the failure of axons to regenerate back into the CNS.

Chondroitinase ABC (ChABC) is a bacterial enzyme that degrades the inhibitory glycosaminoglycan (GAG) chains of chondroitin sulfate proteoglycans (CSPGs) found in the glial scar. In recent experiments, treatment with ChABC allowed for strong penetration of regenerating axons back into the CNS from a peripheral nerve bridging a C3 lesion, resulting in significant behavioral improvements in the ipsilateral forelimb (Houle, et al., 2006, Tom and Houle, 2008, Tom, et al., 2009). Treatment with ChABC is required because of the rapid deposition of CSPGs at the site of CNS trauma and bridge insertion near the vicinity of the denervated target neurons. Additionally, CSPGs are a component of the perineuronal net, which increases around denervated neurons, including motor neurons well away from the site of damage. The appearance of CSPGs within the perineuronal net during development coincides with the cessation of developmental plasticity (Pizzorusso, et al., 2002, Pizzorusso, et al., 2006). In CNS trauma, it has been shown that CSPGs strongly inhibit regeneration and that plasticity through ChABC treatment can promote behavioral recovery (Barritt, et al., 2006, Bradbury, et al., 2002, Busch and Silver, 2007, Cafferty, et al., 2008, Massey, et al., 2006, Silver and Miller, 2004, Steinmetz, et al., 2005, Tester and Howland, 2008). In our own experiments we are currently using ChABC treatment alone and in conjunction with peripheral nerve grafting to promote strong recovery of respiratory function. Briefly, there is an increased presence of lesion scar, as well as perineuronal net associated CSPGs, at the level of the phrenic nucleus, which powerfully inhibit regeneration/plasticity of respiratory function. Degradation of the glial scar allowing for regeneration can promote recovery, which is augmented by potential modification of the inherent spinal circuitry and sprouting of spared tracts (Alilain et al., 2006, Alilain et al., 2007 and Alilain et al., 2011)

The quick and intense reaction of the CNS to trauma in particular, the increased presence of CSPGs, makes natural regeneration of severed axons near impossible. In addition to promoting sprouting and regeneration of essential tracts to respiration to promote recovery, manipulation of the spinally located respiratory neurons is another strategy to restore function.

Plasticity and modification of respiratory spinal neurons

Paralysis of the hemidiaphragm following C2 hemisection is often a transitory condition. At chronic time points following injury, there is normally a very modest return of respiratory

function (Nantwi, et al., 1999, Pitts, 1940, Fuller, et al., 2008). Coinciding with this spontaneous return of activity are synaptic changes in or near the phrenic motor pool (mentioned earlier in this review) including plasticity of the serotonergic and excitatory glutamatergic systems.

For the serotonergic system, at chronic time points there is an increase of presynaptic 5-HT terminals from the caudal Raphe nucleus as well as an increase in its receptors, in particular the 5-HT 2A subtype (Fuller, et al., 2005, Golder and Mitchell, 2005, Tai, et al., 1997). In addition to its effect on LTF, as noted earlier in this review, an increase in serotonergic neurotransmission is important because it has been shown that 5-HT is a requirement for the induction of the crossed phrenic phenomenon itself. Depletion of 5-HT results in abolishment of the morphological changes associated with the phenomenon and failure to induce it (Hadley, et al., 1999, Hadley, et al., 1999). Treatment with 5-HT receptor agonists is also sufficient to induce activation of the crossed phrenic pathway and restore function (Ling, et al., 1994, Zhou, et al., 2001, Zhou and Goshgarian, 1999, Zhou and Goshgarian, 2000, Zimmer and Goshgarian, 2006). While 5-HT may have a modulatory role in respiratory motor output, glutamate from the rVRG is the excitatory neurotransmitter that directs the phrenic motor neuron to fire (Chitravanshi and Sapru, 1996). Similar to 5-HT, at chronic time points there is an increase in glutamatergic terminal length (Tai and Goshgarian, 1996). For the glutamate receptors, an increase in the 2A subunit of the NMDA receptor and a decrease of the AMPA GluR1 subunit on phrenic motor neurons correlates with the onset of spontaneous activity (Alilain and Goshgarian, 2008). This finding is also observed in other models of SCI (Grossman et al., 1999, Grossman et al., 2000). Furthermore, pharmacologically increasing the 2A subunit can result in recovery with the NMDA receptor antagonist MK-801 through “disuse hypersensitization”. This treatment has unwanted side effects on the animals, but suggests the importance of this receptor subunit (Alilain and Goshgarian, 2007). Overall, similar to the series of experiments examining LTF and plasticity of phrenic output, the same elements of 5-HT and glutamate neurotransmission play major roles in mediating recovery after injury.

The role of interneurons in respiratory plasticity

In addition to the phrenic motor neurons, there have been recent studies regarding the importance of pre-phrenic spinal interneurons in the spontaneous recovery process (Fig. 1). A recent elegant series of experiments showed that there are a number of different types of pre-phrenic interneurons located bilaterally between the brainstem and the SC (Lane, et al., 2009, Lane, et al., 2008). In the unlesioned animal, these interneurons can potentially enhance or amplify phrenic output (Hayashi, et al., 2003). In the injured animal at time points where spontaneous recovery occurs, these interneurons may play a role in mediating spontaneous recovery. Recent studies suggest either recruitment or strengthening of these cells (or possibly both) and a capacity of the CNS respiratory circuitry to adapt and reorganize in response to injury. These interneurons, when harnessed, can play an important and complex role in mediating recovery. Optogenetic control of this interneuron population is discussed below. Other experiments attempting to utilize these interneurons and the spinal phrenic circuitry include fetal/donor cell transplants and grafts directly into the area near these cells to potentially act as a cell replacement or influence on the spinal respiratory circuitry (White, et al. 2010). Indeed, the results have shown an impact on respiratory output; however, more work still needs to be done regarding this approach.

Optogenetic approaches to induce plasticity of lesioned respiratory pathways

With the emergence of the light sensitive cation channel, channelrhodopsin-2 (ChR2), new ways of precisely controlling CNS activity are now possible (Herlitze and Landmesser, 2007, Zheng et al 2007). In SCI, which can result in denervated neurons, this tool becomes immensely useful. Expression of ChR2 and subsequent photostimulation can lead to neuronal depolarization and/or induction of action potentials, independent of presynaptic input. Indeed, it was shown that when stimulated with light, ChR2 expressing spinal cells at the cervical level after C2 hemisection can lead to a near complete restoration of respiratory activity which persisted long after the cessation of light exposure (Alilain, et al., 2008, Alilain and Silver, 2009). Perhaps more interesting, the activity that was evoked in the hemidiaphragm ipsilateral to the lesion was not entrained to the pattern of light stimulation (0.5 Hz) but was synchronized to the contralateral side. These results suggest that repeated photostimulation and spinal cell depolarization can potentiate these cells to spared and weak pathways with sparse neurotransmitter release, in this case the crossed phrenic pathway.

Interestingly, the cell types that expressed the construct included both motor neurons and interneurons (as well as some astrocytes). Furthermore, recovery was achieved through a unique pattern of oscillating waves between both sides of the diaphragm, although ChR2 was expressed on only one side. Potentially serving as the anatomical substrate for this bilateral phenomenon were both interneuron and motor neuron populations with neurite extensions projecting toward the contralateral side of the SC (Fig. 1). When MK-801 was administered immediately prior to photostimulation, the induction of the oscillating phenomenon and restoration of activity was totally abolished - indicating an important role of the NMDA receptor and its ability to facilitate plasticity. These results clearly provide further data demonstrating the potential of the inherent plasticity of the spinal respiratory circuitry. Because this experiment utilized the general cytomegalovirus (CMV) promoter, it will be interesting to see whether future experiments, employing more specific promoters, will define which cell types contribute to the return of function.

With regards to future optogenetic approaches to manipulate spinal cord plasticity, there are many strategies. Combinatorial treatment with ChABC, as well as, photostimulation of supraspinal centers which directly influence phrenic motor output including the raphe nucleus and rVRG could potentially enhance the plasticity of the spinal cord and augment recovery.

Other models of SCI with respiratory complications

While the C2 hemisection model of SCI allows for investigation of spared nerve pathways and the reinnervation of the phrenic nucleus, comparatively little has been done about restoring function to the accessory muscles of respiration. These muscles act in concert with the diaphragm not only in rhythmic breathing but also in augmented functions such as sighing, yawning, sneezing, and coughing. The internal intercostals and abdominal muscles (T1–T11) are the accessory muscles of expiration. The external intercostals (T1–T11), scalenes (C4–C6), and sternocleidomastoid (CN XI) are the accessory muscles of inspiration.

It is important to study these muscles and the effect SCI has on them, because: 1) optimal diaphragm function depends on its ability to act in concert with muscles of the thoracic cage and abdominal wall, 2) augmented inspiration and forced expiration are independent of diaphragmatic function and essential for sighing and coughing, and 3) many actual incidents are thoracic SCIs which do not involve denervation of the phrenic nucleus but still impose

respiratory insufficiency. The methods and applications for investigating intercostal and abdominal respiratory function are discussed below.

One must consider that the C2 hemisection model cannot be advanced to a full transection thereby affecting all respiratory related muscles, as it is essentially impossible to model a ventilator dependent human in an animal model. Primarily due to logistical issues, it cannot be applied to higher vertebrates and non-human primates (Philips 2004). Lower cervical or upper thoracic injury, in contrast, preserves function of the diaphragm while still allowing study of respiratory dysfunction and the plasticity of mammalian models similar to humans such as dogs (DiMarco et. al. 1995, Kowalski et. al. 2007, Walter et. al. 2010). One method involves contusion of the spinal cord at T8. With a T8 contusion there ensues a significantly decreased tidal volume and an increased respiratory rate in the rat model (Teng et. al. 1999, Teng et. al. 2003). Perhaps, not surprisingly, 5-HT also plays a role in reversing respiratory deficits in this particular model (Teng, et al., 2003, Teng, et al., 1999). More recently, contusion injuries at the cervical level are being investigated. For more on the contusion model at the C2 level please see the article by Lane et al., in this issue and the recent article by Golder et al. (2011). Furthermore, besides being more clinically relevant in terms of animal model, utilization of the contusion injury, at any level, may help to translate the model to actual injuries encountered by humans. Other models use a full transection in cats and dogs. In an interesting study by DiMarco et al., they demonstrated that in addition to activating expiratory muscles and large positive airway pressures, stimulation of the SC at caudal thoracic levels can lead to activation of diaphragmatic activity before and after C2 section, clearly demonstrating the plastic nature of the circuitry of the SC, at least acutely, which can be harnessed for restoration of activity (DiMarco et. al., 1999, DiMarco et. al., 2008).

Transition from the laboratory bench to the bedside

It is clear from these experiments, the rat provides an excellent model for respiratory motor dysfunction, CNS plasticity, and repair after spinal cord injury. However, there are some limitations. It has been well established by patient models that diaphragm function alone is not enough to sustain life and quality of life in patients with SCI who also have an impaired ability to augment their inspiratory capacity in response to the hypoxic state: individuals are mechanically ventilated which only allows rhythmic activation of diaphragm contractions (DiMarco 2005, Sander et. al., 2010). Although restoration of diaphragm activity following SCI is of high importance, there are other issues that need to be considered. The diaphragm's contractile properties are not sufficient to restore full inspiratory capacity; adequate ventilation requires simultaneous contraction of the external intercostals to allow adequate lung expansion during inhalation (Walter et. al., 2010, Philips 2004). In addition, without periodic stretching of the ribcage in breathing, the joints themselves become calcified and rigid, resulting in reduced ventilation and expedited diaphragmatic fatigue. Furthermore, the intercostal muscles are essential for deep inhalation to relieve atelectasis in an able-bodied individual; normal respiration would need restoration of the periodic sigh provided by external intercostal contraction.

Forceful expiration may be an even more important role for accessory muscles of respiration (DiMarco et. al, 1995). Many studies describe forced expiration as the ability to react to stimuli irritating the respiratory tract, as in a cough (Lim et. al. 2007, Kowalski et. al. 2007, Jefferson et. al. 2010). Cough allows for expulsion of harmful stimuli and possible pathogens, of which the SCI patient is already at potential risk (Dimarco et. al. 2009). When the coughing mechanism is suppressed, probability of upper respiratory infection in the already immunocompromised patient is a certainty. These same studies that emphasize the importance of the muscles of expiration (the internal intercostals and abdominals) are

attempting to develop electrical stimulation to restore impulses to these muscles based on the principle of muscle priming to restore automatic function (Jefferson et. al., 2010, Lee et. al, 2008, Martin et. al, 1992, Schilero et. al. 2009) or stimulation upon command (Lim et. al., 2007, Sander et. al., 2010). For instance, DiMarco et. al. 2002 and DiMarco 2008 successfully produced positive airway pressure in both the dog and cat model by stimulating spinal cord levels between T9 through T10 to generate a cough. Such promising studies support the importance of a holistic approach to restoration of muscle function in spinal cord injury.

In concert with the notion that phrenic innervation is not the only goal of SCI respiration recovery is the idea that many patients are either ineligible for phrenic nerve pacing or need support other than diaphragmatic innervations. As described by DiMarco et. al, patients with unilateral phrenic nerve function may not be able to adopt phrenic nerve pacing because of the remaining functioning hemidiaphragm. However, because cough is still suppressed in these individuals, new techniques such as functional electrical stimulation, surface stimulation, or magnetic stimulation may be able to isolate expiratory muscles to preserve cough (DiMarco et. al. 2005).

Conclusions

As one can clearly see, there has been much progress in investigating potential therapeutic strategies to restore respiratory function after SCI. This includes uncovering an examination of the inherent plasticity of the spinal cord and respiratory activity, which can be harnessed and exploited to restore function after injury. However, there is still much work to be done in further characterizing these processes and the basic science behind these mechanisms. Potentially in the end, a multi-faceted strategy that combines many of the described approaches in this review will fully restore respiratory function and aid in improving the outcomes of the SCI community and their quality of life.

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Figure 1. A schematic of the medullary-spinal cord respiratory circuitry

From the brainstem, bilateral projections are made from the RVRG (green) to the phrenic nucleus (red), providing the inspiratory drive. These pathways decussate at the medulla, as well as at the cervical spinal cord, which comprises the crossed phrenic pathway and is normally latent. Following injury at the cervical level, these pathways become interrupted resulting in impaired diaphragm activity. Furthermore, these bulbospinal projections are not exclusively monosynaptic, with interneurons dispersed in the circuitry (blue). These interneurons have become a target to restore function after injury.