Respiratory plasticity following spinal cord injury: perspectives from mouse to man

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Abstract

The study of respiratory plasticity in animal models spans decades. At the bench, researchers use an array of techniques aimed at harnessing the power of plasticity within the central nervous system to restore respiration following spinal cord injury. This field of research is highly clinically relevant. People living with cervical spinal cord injury at or above the level of the phrenic motoneuron pool at spinal levels C3–C5 typically have significant impairments in breathing which may require assisted ventilation. Those who are ventilator dependent are at an increased risk of ventilator-associated co-morbidities and have a drastically reduced life expectancy. Pre-clinical research examining respiratory plasticity in animal models has laid the groundwork for clinical trials. Despite how widely researched this injury is in animal models, relatively few treatments have broken through the preclinical barrier. The three goals of this present review are to define plasticity as it pertains to respiratory function post-spinal cord injury, discuss plasticity models of spinal cord injury used in research, and explore the shift from preclinical to clinical research. By investigating current targets of respiratory plasticity research, we hope to illuminate preclinical work that can influence future clinical investigations and the advancement of treatments for spinal cord injury.

Key Words: breathing; phrenic; plasticity; rehabilitation; respiration; spinal cord injury; translation

Introduction

Each year there are more than 17,000 new spinal cord injury (SCI) cases in the United States and an estimated 294,000 people living with some level of SCI today (National Spinal Cord Injury Statistical Center, 2020). The most common location of traumatic injury to the spinal cord is the cervical region (National Spinal Cord Injury Statistical Center, 2020). Injury to the spinal cord disrupts ascending and descending neural pathways and can affect motor and sensory function. Injuries occurring in people are complicated and heterogeneous, varying substantially in neuropathology. People living with cervical SCI at or above the level of the phrenic motoneuron (PHMN) pool (cervical levels C3–C5) typically have significant impairments in breathing which may require assisted ventilation. Respiratory input from the brainstem innervates the spinal phrenic network controlling the diaphragm (the primary muscle of inspiration) and more causal motor networks that control intercostal and abdominal muscles (Figure 1). Direct damage to the phrenic network, loss of supraspinal drive to this network, and denervation to more caudal circuits (intercostal and abdominal) result in associated muscle paresis or paralysis and subsequent muscle atrophy. Such injuries not only usually necessitate assisted ventilation, but these individuals have a higher risk of secondary complications including pneumonia, may suffer from additional deficits including impaired cough reflex, impaired mucociliary clearance, and sleep-disordered breathing (Baydur and Sassoon, 2010; Chiody et al., 2016; Sankari et al., 2019). The National Spinal Cord Injury Statistical Center reports that the life expectancy of a 20-year-old, ventilator-dependent patient falls from 59.4 years with no injury to just 10 years post-injury (National Spinal Cord Injury Statistical Center, 2020). With this in mind, research centered on the restoration of independent breathing and improvement in quality of life has become a nexus of both clinical and biochemical research.

The very earliest clinical case reports from ancient Egypt recognized the brainstem as an essential component of breathing (Imhotep, 2650 BC). In the mid 1800s, Flourens (1858) also suggested that the “noeud vital” (vital node) for respiration was located between the V of the grey matter in the medulla. This work was supported by funds awarded from the National Institutes of Health R01 NS104291 and Wings for Life (to MAL); the Lisa Dean Moseley Foundation (to LVZ). Correspondence to: Michael A. Lane, PhD, mlane.neuro@gmail.com.

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This network and plasticity via the CPP help to explain spontaneous contraction of a previously paralyzed hemidiaphragm following SCI seen both clinically and in research. In 1868, Brown-Sequard noted that a hemi-lesion between C1–C4 did not disrupt the diaphragm (Brown-Sequard, 1868). In 1895, Porter eventually confirmed the findings and systematically documented the CPP in a pre-clinical model of complete C2 Hemisection (C2Hx) (Porter, 1895). It is important to note that the cases Brown-Sequard reported on were likely neurologically complete lesions, but also anatomically incomplete hemi-lesions (although often misleadingly represented as anatomically complete). For 56 years the CPP went relatively unexplored until 1951 when Lewis and Brookhart published work concluding that the extent of functional recovery attributable to CPP varies in proportion to the discharge from supraspinal respiratory centers (Lewis and Brookhart, 1951; Goshgarian, 1999). Since then, this system has evolved to include inducible CPP by methods such as hypoxia, hypercapnia, and asphyxia (Lane et al., 2008a, 2009, Goshgarian, 2009).

In addition to these examples of phrenic plasticity, similar spontaneous improvements have been seen in other respiratory networks such as the intercostals, parietal pleura, or thoracic parietal pleura. Pre-clinical studies revealed spontaneous improvement in intercostal function weeks after a high cervical SCI (Dougherty et al., 2012). This functional plasticity was attributed to a “crossed-intercostal” pathway (Dougherty et al., 2012), which may consist of direct input from brainstem respiratory centers or polysynaptic pathways with spinal interneurons that are known to exist within these spinal networks (Lane et al., 2008b). Progressive recovery of cough has also been shown following thoracic SCI in the cat model (Jefferson et al., 2010). Compared to the evidence for plasticity within the phrenic network, less is known about the intercostal and abdominal respiratory pathways, which networks contribute to plasticity, and how it translates to changes in ventilation or recovery from injury. Previous respiratory research following thoracic SCI showed that spontaneous plasticity is limited and deficits after SCI persist in both preclinical models (Kowalski et al., 2007) and the human population (Brown et al., 2006; Baydur and Sassoon, 2010).

While injury at cervical, thoracic, and lumbar spinal levels can compromise respiratory networks, the most devastating consequence arises following cervical injury, which is the focus of the remaining part of this review. Cervical SCI can lead to respiratory failure and several secondary complications making them targets of therapy vast. The three goals of this review are to define plasticity as it pertains to respiratory function post-SCI, discuss plasticity models of SCI used in research, and explore the shift from preclinical to clinical research. By investigating current targets of respiratory plasticity research, we hope to illuminate preclinical work that can influence future clinical investigations and the advancement of treatments for SCI.

### Search Strategy and Selection Criteria

PubMed database was used to search for all references cited in this manuscript, and citations were added using Endnote software. References were searched based on known content or using search terms relevant to the statements being made. No exclusion criteria were used in searches.

### Choosing a Pre-Clinical Model of Spinal Cord Injury

Besides considering the spinal level of injury (e.g., cervical vs. thoracic), the type of injury used to model SCI can also have important differences in neuropathological and functional outcomes (Table 1). The most frequently used model of respiratory dysfunction and plasticity after SCI is the lateral C2Hx (Goshgarian, 2003; Vinits and Kastner, 2009; Hoh et al., 2013; Warren et al., 2014, Figure 2). Given the early studies by Porter in adult canines, the C2Hx model has essentially been used for almost 130 years (Porter, 1895). This spinal injury model has been used to study respiration and neuroplastic potential (see below) post-SCI in a number of species, most commonly rats (Golder et al., 2001; Fuller et al., 2008, 2009; Lane et al., 2008b; Lee et al., 2013) and mouse (Minor et al., 2006; Seeds et al., 2009; Zhuhdeva et al., 2017; Satkunendrarajah et al., 2018; Michel-Floutot et al., 2021a).

In more recent years, a growing number of studies have used a clinically comparable contusion injury model (Kwon et al., 2002; Scheff et al., 2003), instead of the C2Hx. This injury model damages both spinal respiratory circuitry (moto- and interneurons) and descending respiratory axons from the VRC to the medulla. In the first to show that lateral or midline cervical contusion resulted in reduced phrenic motor output and attenuated phrenic motor response to the respiratory challenge. For example, a lateral contusion injury between cervical levels 3 and 4 (C3/4) disrupts the descending respiratory bulbospinal axons and damages both grey and white matter within the cervical spinal cord. The resulting neuropathology and asymmetric lesion are more comparable to injuries usually occurring in people (Saito and Baydur, 2003; DMarc, 2009; Alarcon et al., 2013), and may even be more comparable to the injuries originally described by Brown-Sequard (Figure 2). Contusive damage typically leads to cystic cavity formation in most mammalian species including humans (Backe et al., 1991; Kwon et al., 2002; Scheff et al., 2003; Lane et al., 2008a, 2012; Talekar et al., 2016; Burks et al., 2019), which are present at mid-cervical levels result in a loss of phrenic moto- and interneurons at the lesion epicenter and denervation of phrenic nerve caudal to injury (Figure 3). This cervical contusion model uses a birth-dated rat, which may have smaller mammalian rodent models, with a translational goal of progressing research to larger models. Considerations in the choice of model begin with the primary questions being asked (and model relevancy), with considerations for network similarities, size of the animal and spinal cord, inflammatory responses to trauma, behavioral patterns, genetic differences.

### Table 1 | Mouse to man: considerations for translation

| Human SCI | Pre-clinical animal models |
|-----------|----------------------------|
| Injury | Highly heterogeneous injuries with varying degrees of damage (e.g., contusion, compression, contusion and root avulsion), which vary in proportion to the discharge from supraspinal respiratory centers (Lewis and Brookhart, 1951; Goshgarian, 1999). Since then, this system has evolved to include inducible CPP by methods such as hypoxia, hypercapnia, and asphyxia (Lane et al., 2008a, 2009, Goshgarian, 2009). |
| Species | The human spinal cord is much larger than most animal models used. Opioid primates, sheep, canine, and feline are among those that are closer to human anatomy. The extent to which underlying neural networks differ between human and non-human species is not entirely clear, but there may be important differences to consider. |
| Post-injury management and treatment | There is high variability in post-injury management that is impacted by varying degrees of compliance with the standard of care and evidence-based guidelines, as well as health care resource accessibility. Management often primarily focuses on optimizing the individual that may have undergone multiple other traumas in addition to the SCI. Given the complexity of SCI patients, patients may or may not undergo surgical intervention. Once stable, patients may undergo surgical decompression and/or arthrodesis. The time before a patient receives surgical intervention or additional medication, or is transferred for rehabilitation, will vary substantially based on socio-economic and health care resource factors (impacted by geographical location and insurance coverage). |
| Outcome measures | Neurological testing and scoring based on ASIA/NSCSCI scales are most common and highly standardized, however, these scales are less effective at evaluating thoracic SCI. Currently, the only standard for evaluating thoracic SCI is the thoracic injury severity score (TISS), which is a quantitative measure of injury. Other outcome measures, such as imaging and electrophysiological, have been shown to have some predictive value but are not considered standard of care. However, there is a lack of consistency in outcome measures employed in clinical trials and there is no approved or mandated “standard.” Accordingly, comparing results between trials can be extremely difficult. |

Injuries are carefully controlled with a goal of reproducibility and consistency, and include thoracic SCI (e.g., contusion, compression, or “laceration”) to a specific region of the spinal cord, affecting known neuronal networks. Pre-clinical models are rapid, single-time-injury that does not include the ongoing damage risks that are seen in people. Often smaller mammalian rodent models, with a translational goal of progressing research to larger models. Considerations in the choice of model begin with the primary questions being asked (and model relevancy), with considerations for network similarities, size of the animal and spinal cord, inflammatory responses to trauma, behavioral patterns, genetic differences.

Some under-appreciated variability. Often considered standardized to limit variability and animal management, the care and treatment that pre-clinical animals receive follow institutionally approved veterinary care. This varies substantially depending on species and may vary by country. Animals are kept warm acutely post-injury and given analgesics immediately. Ongoing treatment regimens (daily analgesics, antibiotics) can vary greatly between investigators, even within an institution. Note: in order to perform most injuries, a laminectomy is performed pre-injury (perhaps comparable to a "pre-injury decompression surgery"). *Dose and timing may vary depending on institutional guidelines, with considerations for what effects it might have on compromised functions like breathing.

Functional/behavioral outcome measures are highly variable between investigative teams and may depend on the level of research funding and access to necessary facilities. A greater understanding of collaborative data sharing and collaboration has helped to address this issue. With the greater drive in the field for transparency, rigor, and reproducibility, the quality of data being reported is improving.
Given that contusive injuries span longer rostro-caudal differences, the extent of disruption and neuronal loss, and the contribution of spared pathways to plasticity may vary between injury models. Using a dual contusion and hemisection model, Allain and colleagues (2011) demonstrated that crossed phrenic nerve stimulation contributes at least partially to the motor function after injury (Awad et al., 2013). Retрогrade, transneuronal tracing of the phrenic motor network also reveals an increase in the number of labeled phrenic spinal neurones (SpNs) connected with the motor network on the side of a contusive injury, highlighting a role for SpNs in plasticity post-injury (Lane et al., 2012).

### Spinal Interneurons and Respiratory Plasticity after Spinal Cord Injury

Recent studies have shown that pre-spinal SpNs, that innervate the phrenic and other respiratory networks, are found throughout the neural axis, and receive input from neurons in the VRC and other respiratory-related supraspinal nuclei (e.g., serotonergic axons from the raphe, several reticular nuclei including the gigantocellular nucleus) (Zholudeva et al., 2018a). While the contribution of these SpNs to breathing remains elusive, several studies have now shown that they modulate respiratory output, integrate the phrenic networks on each side of the spinal cord, and integrate phrenic with other respiratory networks (Lane et al., 2008a, 2009; Lane, 2011; Darlot et al., 2012; Buttry and Goshgarian, 2015; Zholudeva et al., 2018a; Jensen et al., 2019). They do so under normal, eupneic breathing, and can change their contribution to output with changing conditions (e.g., hypoxia) (Lane et al., 2009; Sandhu et al., 2009; Streeter et al., 2017). Even more pertinent to the present discussion, these cells have been shown to contribute to both spontaneous and therapeutically driven plasticity post-SCI. SpNs are being increasingly considered to be key therapeutic targets for promoting functional recovery (Harkema, 2008; Zholudeva et al., 2018a, 2021; Zavvarian et al., 2020).

### Enhancing Respiratory Neuroplasticity after Spinal Cord Injury

Neuroplasticity is the ability of the central nervous system to change either anatomically or functionally (or both) resulting in persistent alterations in neural output and function. This ability is an integral part of normal physiology and, following injury, can be either adaptive or maladaptive (Table 2). The CPN, as described above, and the plasticity that has been reported following contusive injury, have provided a window to preclinical data on adaptive plasticity within the phrenic motor network. Moreover, clinical reports of progressive respiratory improvement following traumatic SCI are beginning to highlight similar examples of plasticity among the human population. Several strategies including neural interfacing, physical stimuli, and growth-promoting agents (pharmacological or genetic) have been identified as promising ways of enhancing neural plasticity and are subject of ongoing research. We will now discuss the targets of SCI research as they apply to spontaneously occurring and therapeutically driven neuroplasticity.

#### Table 2 | Types of functional plasticity

| Plasticity within neural networks: General changes in neural output | Adaptative plasticity | Restorative | Compensatory | Maladaptive plasticity |
|---|---|---|---|---|
| Plasticity function in respiratory circuits | Restoration of function | Altered activity within respiratory circuits (and the muscles they control) | The amplitude or pattern of neural output may become dysfunctional (e.g., weakened or arhythmic), limiting recovery or contributing to the deficit. | The onset of inappropriate patterns of ventilation prior to injury. |
| Plasticity within respiratory nuclei | Restoring the ability to perform ventilation in exactly the same manner | Effective ventilation, but performed in a manner different from how it was performed prior to injury (e.g., rapid, shallow breathing) | | |
| Maladaptive plasticity | | | | |

The following table provides the definitions for types of plasticity that occur either within neural networks or at a behavioral level. Modified from Klein (2013) and Hromig et al. (2017).

### Neural interfaces

Stimulation of neuronal networks via neural interfaces (e.g., electrical stimulation) activates spared networks and contributes to modest anatomical and functional plasticity. These stimulation strategies include epidural, intraspinal, functional electrical stimulation, and transcranial magnetic stimulation (Edgerton and Harkema, 2011; Young, 2015; Hormigo et al., 2017; Courtine and Sofroniew, 2019; Zavvarian et al., 2020; Figure 4A). The last decade has seen rapid advances in the engineering of hardware for interfacing with the injured spinal cord (Courtine and Sofroniew, 2019). With the development of more biocompatible materials, invasive implantation is becoming more translatable. The resolution at which we can stimulate a given number of pixels and the precision of respiratory rate control is improving. Simultaneously, preclinical research has seen a rapid advancement in the development of less invasive hardware, including optrodes for epidural stimulation of neural tissues (Mondello et al., 2018). Coming years may also see the preclinical development of non-invasive optogenetic, chemogenetic, and ultrasonic capabilities for activating injured networks. Within the respiratory networks, a host of neural interfacing strategies have been explored, including diaphragm stimulation (DiMarco et al., 2015; Orlanders, 2012; DiMarco, 2018), intraspinal magnetic stimulation (DiMarco et al., 2005a; DiMarco and Kowalski, 2010, 2015), epidural stimulation (DiMarco and Kowalski, 2009; Kowalski and DiMarco, 2011; Kowalski et al., 2013, 2016, Gonzalez-Rothi et al., 2017; Bezduynaya et al., 2019) and to a lesser extent intraspinal ultrasonic stimulation (Chesnutt et al., 2017). In an effort to develop and characterize less invasive, yet translationally relevant strategies of importance to functional plasticity, clinicians are exploring the use of transcutaneous stimulation (Mitsui et al., 2021). For a more detailed discussion on neural interfacing methods, please see (Orlanders, 2012; DiMarco and Kowalski, 2013; Hormigo et al., 2017).

#### Activity-based therapies

Respiratory training encompasses rehabilitative, resistive, and activity-based training methods to strengthen the neuromuscular respiratory circuitry. These neuromuscular respiratory therapies (e.g., locomotor training or hypercapnia training) as well as non-respiratory strategies such as locomotor training (Randelman et al., 2021; Figure 4B). Resistance-based training strategies have been shown to significantly improve respiratory function in people with a wide range of respiratory disorders (Kenna and Wheeler, 2006), and can assist in weaning from assisted ventilation. As ongoing clinical studies explore how and when muscle strength training can be used, it may become a key component of chronic respiratory strategies for people with SCI. Locomotor training for people with SCI has been shown for decades to improve motor outcomes (Behrman and Harkema, 2000; Harkema, 2001; Barbeau et al., 2006; Edgerton et al., 2006, 2008; Harkema et al., 2012; Behrman et al., 2017) and in more recent years these benefits have been seen across a wider range of functions in both the clinical population (Harkema et al., 2008; Terson de Paleville et al., 2013) and in pre-clinical studies (Ward et al., 2014, 2016; Hubsher et al., 2016; Harman et al., 2021).

One example of a non-invasive respiratory activity-based therapy uses decreased oxygen levels (hypoxia). This form of respiratory training—known as intermittent hypoxia (IH) or intermittent hypoxic training (IHT) or intermittent hypoxia with alternating levels of normoxia. These IH protocols can vary by the severity of hypoxia (e.g., percent of O2), duration of hypoxic episodes, number of hypoxia/normoxia cycles per day, longevity of treatment, and/or start time of treatment following injury (Dale-Nagle et al., 2010; Dale et al., 2014; Gonzalez-Rothi et al., 2015, 2021). One of the hallmarks of respiratory plasticity with IH training is the ability to elicit persistent (hours) increases in phrenic nerve activity called phrenic long-term facilitation (Fuller et al., 2000; Mitchell et al., 2001; Devinney et al., 2013). Many of the IH-facilitated respiratory plasticity pathways are serotonin (Ing et al., 2001; Baker-Herman and Mitchell, 2002; Golder and Mitchell, 2005) and BDNF dependent (Baker-Herman et al., 2004). Studies show that intermittent training with hypoxia can enhance respiratory motor output and tidal volume (Lovett-Barr et al., 2012) and restore breathing capacity after preclinical SCI (Vinit et al., 2009; Navarrete-Opazo et al., 2015; Dougherty et al., 2016).

An alternative form of gas training to hypoxia is to increase levels of carbon dioxide (i.e., intermittent hypercapnia) while maintaining normoxia (Randelman et al., 2021.). While early studies using chronic hypercapnia revealed little efficacy (Bach and Mitchell, 1996, 1998; Baker et al., 2001), intermittent hypercapnia appears to promote some adaptive respiratory plasticity (Baker and Mitchell, 2000; Baker et al., 2001). Combining hypercapnia with hypoxia has also been shown to enhance respiratory function, tidal volume, and frequency after SCI (Lee et al., 2017; Wen et al., 2013, 2020b; Wen et al., 2021). The timing (level of gas) and duration (exposure time) of different types of gas as a "chemical" stimulus is crucial for effectively modulating respiratory plasticity. As preclinical studies continue to refine these therapeutic approaches there will be a strong potential for rapid translation into the clinic.

#### Pro-regenerative treatments

The last two pro-regenerative treatments is typically to enhance axon outgrowth for the repair of neural networks capable of restoring function. The barriers to growth can be broadly divided into intrinsic and extrinsic barriers. Various neuronal intrinsic mechanisms contribute to structural plasticity including gene transcription, synaptic plasticity, axonal guidance, neurotrophic, intrinsic neuronal molecular brakes, and mitochondrial energy deficits. Extrinsic barriers to axonal growth such as cellular damage and death, upregulated matrix molecules (e.g., perineuronal net), myelin and protein deposition, and astrocytic scar tissue also contribute to attenuated neural growth. Both intrinsic and extrinsic mechanisms controlling structural plasticity and axon growth have been targeted with pharmacological and genetic strategies (e.g., viral vectors) and are described in detail elsewhere (Tesdici and Bradke, 2017), and have been applied as anatomical and functional repair strategies for respiratory networks (Nantwi and Goshgarian, 2017).
Cervical spinal cord injury in "mouse" and "man". Models of cervical spinal cord injury and resulting respiratory deficits. Schematic diagram of the cervical spinal cord highlighting the phrenic network, comprising phrenic motoneurons (green), pre-motor spinal interneurons (purple), and descending bulbar input (grey, VRC). These images compare two spinal cord injury (SCI) models that are commonly used to examine respiratory function and plasticity. The C2 hemisection (A) is the most frequently used model of SCI for examining respiratory plasticity after SCI. This model completely disrupts all descending pathways from the ventral respiratory group (VRC) in the medulla, to the phrenic motoneurons (green) on the same side (ipsilateral). Electrophysiological recording from this denervated network shows diaphragm paralysis, but sustained activity on the contralateral side (likely undergoing compensation). In contrast, contusive models of SCI (B) more closely resemble the neuropathological deficits associated with human SCI. Most human injuries also occur at mid-cervical levels denervating some phrenic motoneurons and resulting in loss of others. Recording diaphragm activity in this pre-clinical model reveals deficits ipsilateral to injury. Ongoing research is studying the neuroplastic potential of this injured anatomical substrate and progressive functional changes. dEMG: Diaphragm electromyography; SCI: spinal cord injury; VRC: ventral respiratory column.

Figure 2 | Cervical spinal cord injury in "mouse" and "man". Comparison of the cervical spinal cord in man (A, C) and rodent (B, D). These representations highlight gross morphology of the cervical spinal cord in each species (A, B) and show examples of clinically occurring traumatic spinal cord injury (C), and common pre-clinical models of cervical spinal cord injury used to study respiratory dysfunction (B). MRI of the human spinal cord (inset, C) from patient seen at Shands Hospital, University of Florida, USA. Unpublished data.

Figure 3 | Models of cervical spinal cord injury and resulting respiratory deficits. Though promising in the treatment of respiratory dysfunction, bringing PTEN antagonist peptide from preclinical models to clinical trial would be a dangerous endeavor as PTEN inhibition is directly linked to the development of some cancers (Miliella et al., 2015). Therefore, longitudinal studies evaluating residual effects of PTEN antagonist peptides are necessary prior to application in humans. Alternatively, there may be other means of targeting such pathways. There is mounting evidence to suggest that activity-based therapies may be a viable non-pharmacological alternative to targeting the mechanisms of enhanced axonal growth (Liu et al., 2012; Gutierrez et al., 2013).

Another strategy that can target both intrinsic and extrinsic mechanisms of repair is cell or tissue transplantation. Within the respiratory networks, preclinical studies have explored the reparative potential of olfactory ensheathing glia (Polentes et al., 2004; Stamegna et al., 2011; Stamegna et al., 2018), fetal spinal cord tissue (White et al., 2010; Lee et al., 2014; Spruance et al., 2018), and their more-refined counterpart neural progenitor cells (Zhuludeva et al., 2018b; Goullo et al., 2019), and peripheral nerve bridges (Gauthier and Lammari-Barreault, 1991; Gauthier and Lammari-Barreault, 1992; Decherchi and Gauthier, 2002; Decherchi and Gauthier, 2002; Allain et al., 2011). While diverse in their mechanism of action (and elusive for some), each of these approaches has demonstrated repair within phrenic motor networks and improvements in respiratory function. Although untested in the clinical arena for respiratory functions, ongoing pre-clinical studies continue to refine and optimize transplantation strategies and improve their translational potential (Charsar et al., 2017; Lane et al., 2017; Zhuludeva and Lane, 2019; Fischer et al., 2020). Despite what is quickly becoming a remarkable potential, translating cell therapies are not without difficulties (discussed in Fischer et al., 2020). A major hurdle faced in translating cellular therapies to the clinical population has been producing large enough numbers of purified human cells in a good manufacturing practice facility, that are identical to those that were shown to have pre-clinical efficacy. Maintaining the survival of donor cells (when needed for the proposed therapeutic effects) is another difficulty that is not easily overcome without significant immunosuppression, and it remains unclear as to how long that is required. Ongoing pre-clinical research is broadening its focus to start exploring some of these hurdles in the hope of paving a smoother translational path.
From Mouse to Man: Plasticity in Clinical Research

The medical management of SCI is constantly evolving. In recent years, clinicians have begun bringing the discoveries of benchwork scientists into clinical research and practice. Particular emphasis has been placed on using exercise as a means to stimulate plasticity with the use of locomotor training. This approach has been shown to improve respiratory function after traumatic SCI, with some limited effort to translate these to the clinical arena, but attaining the greatest degree of improvement will likely require a combination of these therapeutic approaches. Each target unique aspects of neural repair and/or the neuromuscular potential of compensatory mechanisms. As the science moves closer to the optimal therapeutic outcomes, future work can start to consider how they can be combined for synergistic effects.

Corticospinal motor neuronal stimulation-sham-acute IH, and then measured respiratory function (Tester et al., 2014; Sutor et al., 2021). While IH has been more readily translated clinically, hypercapnia training (intermittent or sustained exposures) has also been demonstrated to evoke respiratory plasticity and ventilatory long term facilitation with and without exposure to hypoxia (Harris et al., 2008; Griffin et al., 2012; Sankey et al., 2015; Bascom et al., 2016; Vermeulen et al., 2020; Welch, 2021). With ongoing clinical use of hypercapnia and mounting evidence for hypercapnia demonstrating therapeutic efficacy in pre-clinical models of SCI, coming years may see this as a newly translated treatment of individuals with injury.

Activity-based or training strategies, pharmacological interventions have also been explored as means for improving respiratory function after SCI. Theophylline is a phosphodiesterase inhibiting drug commonly used in the treatment of chronic and acute pulmonary disease, i.e., increased cardiopulmonary activity (diaphragmatic activity) during and after injury. It is theorized that theophylline improves respiratory muscle strength by enhancing descending bulbospinal pathways and/or increasing the intrapulmonary fatigue of the skeletal muscle in the diaphragm, intercostal, and transversus abdominis muscles (Lim et al., 2021). Theophylline has previously been shown in animal models to restore phrenic nerve activity by inhibiting the adenosine A1 and A2-receptors (Nantwi and Goshgarian, 1998, 2002) and activating quiescent tracts in the contralateral spinal cord via the inducible cyclic AMP response element-binding protein (CREB). Moreno et al. (1999) have shown significant improvements in animal models, clinical trials have failed to find a significant improvement in pulmonary function following treatment protocols of oral theophylline (Moxham et al., 1985; Zepelis et al., 2006) though there is some evidence to support the hypothesis that increased rates of ventilatory function in normal patients can be attributed to theophylline use (Zakrasek et al., 2017).

Another therapeutic strategy that has shown rapid pre-clinical advances and translation to the clinical arena is the use of the vast array of neural interfacing strategies. The primary therapeutic goal of neural stimulation post-SCI is to restore sufficient activity to denervate spinal motor networks and subsequently to stimulate the diaphragm and other accessory muscles of ventilation by direct electrical stimulation. When applied under the right conditions, the beneficial effects can also persist after treatment termination (Dobkin, 2003; DiMarco, 2005; Courtine et al., 2009; Onders, 2012; Mondello et al., 2014; Poslusny et al., 2014). There are many ways to stimulate the spinal cord, including functional electrical stimulation, intraspinal, epidural, or transcerebrum stimulation, or trans magnetic stimulation (DiMarco, 2005; Martin et al., 2012; Onders, 2012; Tator et al., 2012; Hormigo et al., 2017) (outlined in Figure 4A). Prior studies demonstrate the beneficial effects of these strategies can facilitate patterns of respiratory (Hormigo et al., 2017) and non-respiratory networks (Taccola et al., 2018; Jack et al., 2020).

Within respiratory circuitry, functional electrical stimulation of respiratory muscles, particularly the diaphragm (diaphragm pacing, DP) has been successfully translated and employed for more than 30 years and can facilitate ventilator weaning and respiratory recovery (Onders, 2012; Poslusny et al., 2014). After cervical SCI diaphragm paralysis/paresis often occurs, and appropriate pacing recovery can be started. However, muscle stimulation still requires the sparing of sufficient spinal (lower) motoneurons to achieve muscle contraction, so not all patients with cervical level SCI are eligible. While preclinical assessment and development of functional electrical stimulation-based strategies continues, another approach to respiratory electrical stimulation therapy, including DP, is proving beneficial in eligible patients (DiMarco, 2005, 2018; Onders et al., 2007; Kowalski et al., 2013; Poslusny et al., 2014). A 2018 study by Onders et al. collected data from 92 participants, 5 days post-SCI, and subsequently examined respiratory function, specifically in four-electrode intramuscular DP, and found complete respiratory recovery in 5 participants, with 2 able to have their electrodes removed (Onders et al., 2018). They concluded that early implantation of DP leads to favorable outcomes and improves the quality of life of people with respiratory dysfunction following traumatic SCI. The complete recovery of respiration with the use of DP is evidence of plasticity within the respiratory circuit and further propels clinical SCI research forward.

Locomotor training has also been shown to improve a range of non-motor functions, including a respiratory function for individuals with chronic cervical and thoracic injuries (Terson de Paleville et al., 2013). This improvement in respiratory function is believed to be from increased heart rate and respiratory rate, physical activity, and improvement in the use of accessory muscles during exercise. In the case of treadmill training (Terson de Paleville et al., 2013), however, the extent of respiratory improvement may also be “dose-dependent”. Terson de Paleville saw improvements in respiratory function for subjects who received 60 minutes of stepping on a treadmill for five days a week, a difference of 12 weeks (Terson de Paleville et al., 2013). In contrast, individuals who received passive robot-assisted stepping did not improve cardiopulmonary function (Jack et al., 2011). One limitation in respiratory recovery might be achieving a sufficient increase in the number of people that number may be insufficient for motor- respiratory coupling post-SCI (Sherman et al., 2009). This hypothesis is supported by hindlimb stimulation (a passive event) producing respiratory rhythm entrainment (Iscoe and Polosa, 1976; Morin and Viala, 2002; Potts et al., 2010), which in part helps to prevent respiratory dyscrasias (a frequency of ventilation that is not optimal) before or during locomotion. Because of these data, we believe there are several possible mechanisms by which locomotor training may promote improvements in breathing, better understanding what these are and how to best harness the therapeutic potential of locomotor training will require more pre-clinical and clinical investigation.

A wide range of treatments have been used in pre-clinical studies to improve respiratory function after traumatic SCI, with some limited effort to translate these to the clinical arena, but attaining the greatest degree of improvement will likely require a combination of these therapeutic approaches. Each targets unique aspects of neural repair and/or the neuromuscular potential of compensatory mechanisms. As the science moves closer to the optimal therapeutic outcomes, future work can start to consider how they can be combined for synergistic effects.

Closing Remarks

Respiratory plasticity following cervical SCI is a devastating and widely researched area of SCI. Yet despite how widely researched this injury is in animal models, relatively few treatments have broken through the preclinical barrier (Table 1). This could be partial because of a lack of funding or too few incentives for academic, clinical, and industry professionals alike; or the impracticality and feasibility of translating this preclinical research to the clinic. The studies presented here suggest a very different picture. Several animal studies in SCI show that locomotor training can facilitate respiratory recovery (Hormigo et al., 2017) and cannot be true for clinical SCI. If these findings are translated to the clinic, they can be effective therapeutic targets. Clinicians have been able to use these findings to inform clinical research and intervention to the benefit of patients’ lives (e.g., advances in diaphragm pacing (Onders et al., 2014, 2018), and respiratory training (Christiansen et al., 2021). Not only is it important for benchwork to inform clinical research, but clinicians must also use clinical work to inform their research (reverse translation, a.k.a., bedside-to-benchtop). The interactions between academic, clinical, and industry professionals, and injured individuals, must become more closely intertwined in order to properly research and validate the impact of the lives those living with SCI. The translation spectrum (Figure 5) involves a wide range of scientists and engineers in both academic and industry environments, and clinical professionals, that need to interact in a timely and effective manner with each other, and with those people living with SCI, to better the translational pathway.
This relatively simple schematic diagram summarizes the quite complex translational path or “spectrum” for research in spinal cord injury. Each stage highlights the possible roles for scientists, engineers, and clinical professionals. Those living with spinal cord injury are represented at the center of this spiral, as they should influence the work being done at each stage of investigation. A gradient has been used to indicate the flow between these individuals and groups associated with translation, avoiding direct arrows from one to the next, as the path is typical, not linear, and can move between these groups in both directions. Ultimately, in addition to translating treatments to the population being treated clinically, these changes from ongoing work should be fed back into the pre-clinical and clinical research being done. Hurdles exist throughout the translational path and can vary in how significantly they disrupt movement from one stage to the next; but there is tremendous enthusiasm for improving translation, and greater efforts are being made to better understand (i) how the translational spectrum should exist, (ii) the roles of those involved and how they are/should be involved, and (iii) what steps therapists in the field can take to unify and streamline translation and providing treatment to those people living with spinal cord injury.

Figure 5 | Translational spectrum.

References

Alheid GF, McCrimmon DR (2008) The chemical neuroanatomy of breathing. Respir Physiol Neurobiol 164:3-11.

Alheid GF, McComb WK, McCrimmon DR (2004) Postinsult feedback influences on an expression. Respir Physiol Neurobiol 143:119-134.

Allain WJ, Horn KP, Hu J, Dick TE, Silver J (2011) Functional regeneration of respiratory pathways after spinal cord injury. Nature 475:196-200.

Austin JW, Rowley CW, Fehlings MG (2013) Pathophysiology of spinal cord injury. In: Essentials of spinal cord injury: basic resarch to phrenic motor function. J Appl Physiol (1985) 84:2099-2105.

Awada B, Darley PM, Ramirez MP, Allain WJ (2013) The role of the crossed phrenic pathway after spinal cord injury and a new model to evaluate therapeutic interventions. Exp Neurol 248:398-405.

Bach KB, Mitchell GS (1996) Hypoxia-induced long-term facilitation of respiratory activity is serotonin dependent. Respir Physiol 104:251-260.

Bach KB, Mitchell GS (1998) Hypocapnia-induced long-term depression of respiratory activity requires alpha2-adrenergic receptors. J Appl Physiol (1985) 84:2099-2105.

Bach BA, Betz RR, Mesevazadeh M, Beck T, Clancy M (1995) Post-traumatic spinal cord cysts evaluated by magnetic resonance imaging. Parasitology 119:607-612.

Baker-Herman TL, Fuller DD, Bavis RW, Zabka AG, Golder FJ, Doperalski NJ, Johnson RA, Watters W, Burks JD, Gant KL, Guest JD, Jamshidi AG, Cox EM, Anderson KD, Dietrich WD, Bunge MB, Green WD, Brown R, DiMarco AF, Host JD, Garshick E (2006) Respiratory dysfunction and management in spinal cord injury. Respir Care 51:858-866.

Boulenguez P, Gauthier P, Kastner A (2007) Respiratory neuron subpopulations and pathways potentially involved in the reactivation of phrenic motorneurons after C2 hemisection. Brain Res 1148:96-104.

Brown-Sequet C (1868) Lectures on physiology and pathology of the central nervous system: lecture one on spinal hemicerebra. Lancer doi:10.1001/journal Neuro Physiol 51:853-866.

Burks JD, Grant KL, Guest JD, Jamshidi AG, Cox EM, Anderson KD, Dietrich WD, Bunge MB, Green WD, Brown R, DiMarco AF, Host JD, Garshick E (2006) Respiratory dysfunction and management in spinal cord injury. Respir Care 51:858-866.

Butler J, Goshgarian HG (2014) Injection of WGA-Alexa 488 into the ipsilateral hemisection of the spinal cord and chronically 2-hydroxyethyl orvinene activity-dependent synaptic plasticity in the respiratory motor pathways. Exp Neurol 261.440-450.

Butler JG, Goshgarian HG (2015) WGA-Alexa transynaptic labeling in the phrenic motor system using intratrabecular injection vs intradural injection. J Neurosci Methods 235:141-147.

Chasar BA, Urban MW, Lepore AC (2017) Harnessing the power of cell transplantation to target respiratory dysfunction following spinal cord injury. Exp Neurol 287:268-275.

Charal BA, Brinton MA, Loko K, Chen AV, Ghosh B, Urban MW, Komarovsky S, Krishnakumar M, Rait S, Pasinelli P, Wright MC, Smith GM, Lepore AC (2019) AVBD-29N promotes respiratory axon plasticity and recovery of breathing function following spinal cord injury. FASEB J 33(18):13783-13793.

Chiodo AE, Atin RG, Bauman KA (2016) Sleep disordered breathing in spinal cord injury: a systematic review. J Spinal Cord Med 39:374-382.

Christiansen L, Chen B, Lei Y, Urban MA, Richardson MSA, Dudgea M, Sandhu B, Rymer WZ, Trumbower RD, Mitchell GS, Perez MA (2021) Acute intermittent hypoxia boosts spinal plasticity in humans with tetraplegia. Exp Neurol 335:113483.

Coenin FL (1973) Effects of various lesions on crossed and uncrossed descending inspiratory respiratory pathways in the cervical cord of the rat. J Neurosci 39:589-595.

Courtine G, Sofroniew MV (2019) Spinal cord repair: advances in biology and technology. Nat Med 25:899-908.

Courtine G, Gerasimenko Y, van den Brand R, Wea Y, Musienko P, Zhong H, Song B, Ao Y, Ichiyama RM, Lavrov L, Roy RR, Sofroniew MV, Edgerton VR (2009) Transformation of nonfunctional spinal circuits into functional motor pathways after the loss of brain input. Nat Neurosci 12:1341-1342.

Dalal K, DiMarco AF (2014) Diaphragmatic pacing in spinal cord injury. Phis Med Rehabil Clin N Am 25:619-629, viii.

Daleke DA, Hoffman MS, MacFarlane PM, Saritomito L, Lovett-Barr MB, Vinit S, Mitchell GS (2010) Spinal plasticity following intermittent hypoxia: implications for spinal injury. Ann NY Acad Sci 1198:252-259.

Daleke DA, Ben Mabrouk F, Mitchell GS (2014) Unexpected benefits of intermittent hypoxia: enhanced respiratory and nonrespiratory motor function. Physiology (Bethesda) 29:39-48.

Darlot F, Cayetanot F, Gauthier P, Matarasso V, Kastner A (2013) Electrical activation of inspiratory muscles via spinal cord stimulation. Respir Physiol Neurobiol 183:186-192.

Devinney MJ, Huxtable AG, Nichols NL, Mitchell GS (2013) Hypoxia-induced long-term facilitation of respiratory activity is serotonin dependent. Respir Physiol Neurobiol 183:186-192.

Dougherty BJ, Lee KZ, Gonzalez-Rothi EJ, Lane MA, Reier PJ, Fuller DD (2012) Recovery of respiratory function following diaphragm pacing in spinal cord injury. Respir Physiol 183:186-192.

Dougherty BJ, Lee KZ, Gonzalez-Rothi EJ, Lane MA, Reier PJ, Fuller DD (2012) Recovery of respiratory muscular function following diaphragm pacing in spinal cord injury. Respir Physiol Neurobiol 183:186-192.

Edgerton VR, Courtine G, Sofroniew MV (2019) Spinal cord repair: advances in biology and technology. Nat Med 25:899-908.

Ellenberger HH, Feldman JL (1988) Monosynaptic transmission of respiratory drive to phrenic motor neurons. J Appl Physiol (1985) 84:2099-2105.

Ellenberger HH, Feldman JL (1988) Monosynaptic transmission of respiratory drive to phrenic motor neurons. J Appl Physiol (1985) 84:2099-2105.

Ellenberger HH, Feldman JL (1988) Monosynaptic transmission of respiratory drive to phrenic motor neurons. J Appl Physiol (1985) 84:2099-2105.

Ellenberger HH, Feldman JL (1988) Monosynaptic transmission of respiratory drive to phrenic motor neurons. J Appl Physiol (1985) 84:2099-2105.

Ellenberger HH, Feldman JL (1988) Monosynaptic transmission of respiratory drive to phrenic motor neurons. J Appl Physiol (1985) 84:2099-2105.
Review

Ellenberg HB, Feldman JL, Goshtaghi HG (1990) Ventral respiratory group projections to phrenic motoneurones: electron microscopic evidence for monosynaptic connections. J Comp Neurol 302:707-714.

Feldman JL, Del Negro CA (2006) Looking for inspiration: new perspectives on respiratory rhythm. Nat Rev Neurosci 7:232-242.

Feldman JL, Mitcheson GI, Hunt KJ (2011) The metabolic cost of passive walking during robotics-assisted treadmill exercise. Technol Health Care 19:21-27.

Janczewski WA, Karwacziwa WA (1990) The role of neural connections centered at the cervical spinal cord in determining rhythm and amplitude of respiration in cats and rabbits. Respiration 59:163-175.

Jefferson SC, Tester NJ, Rose M, Blum AE, Howland BG, Bowler DC, Howland DR (2010) Cough followed by low thoracic hemisection in the cat. Exp Neurol 222:165-170.

Jensen VN, Allain WI, Crane SA (2019) Role of propriospinal neurons in control of respiratory muscles and recovery of breathing following frontotopic injury. Front Syst Neurosci 13:141.

Jatbrau P, de Sousa P, Schierholz O, de Bruijne TJ, Steinhoff K, van der Ploeg PH, Bauters M, de Vries HD, Zijlstra W, Zwinderman AH, van der Valk P (2012) Intraplantar injection of bone marrow-derived mesenchymal stromal cells in the injured spinal cord. J Neurotrauma 29:1843-1851.

Jilani T, Preuss C, Sharma S (2021) Theophylline. Treasure Island, FL:StatPearls Publishing.

Jefferson SC, Tester NJ, Rose M, Blum AE, Howland BG, Bowler DC, Howland DR (2010) Cough followed by low thoracic hemisection in the cat. Exp Neurol 222:165-170.

Jansen VA, Allain WI, Crane SA (2019) Role of propriospinal neurons in control of respiratory muscles and recovery of breathing following frontotopic injury. Front Syst Neurosci 13:141.

Jefferson SC, Tester NJ, Rose M, Blum AE, Howland BG, Bowler DC, Howland DR (2010) Cough followed by low thoracic hemisection in the cat. Exp Neurol 222:165-170.

Jansen VA, Allain WI, Crane SA (2019) Role of propriospinal neurons in control of respiratory muscles and recovery of breathing following frontotopic injury. Front Syst Neurosci 13:141.

Jefferson SC, Tester NJ, Rose M, Blum AE, Howland BG, Bowler DC, Howland DR (2010) Cough followed by low thoracic hemisection in the cat. Exp Neurol 222:165-170.

Jansen VA, Allain WI, Crane SA (2019) Role of propriospinal neurons in control of respiratory muscles and recovery of breathing following frontotopic injury. Front Syst Neurosci 13:141.

Jefferson SC, Tester NJ, Rose M, Blum AE, Howland BG, Bowler DC, Howland DR (2010) Cough followed by low thoracic hemisection in the cat. Exp Neurol 222:165-170.

Jansen VA, Allain WI, Crane SA (2019) Role of propriospinal neurons in control of respiratory muscles and recovery of breathing following frontotopic injury. Front Syst Neurosci 13:141.

Jefferson SC, Tester NJ, Rose M, Blum AE, Howland BG, Bowler DC, Howland DR (2010) Cough followed by low thoracic hemisection in the cat. Exp Neurol 222:165-170.

Jansen VA, Allain WI, Crane SA (2019) Role of propriospinal neurons in control of respiratory muscles and recovery of breathing following frontotopic injury. Front Syst Neurosci 13:141.

Jefferson SC, Tester NJ, Rose M, Blum AE, Howland BG, Bowler DC, Howland DR (2010) Cough followed by low thoracic hemisection in the cat. Exp Neurol 222:165-170.

Jansen VA, Allain WI, Crane SA (2019) Role of propriospinal neurons in control of respiratory muscles and recovery of breathing following frontotopic injury. Front Syst Neurosci 13:141.

Jefferson SC, Tester NJ, Rose M, Blum AE, Howland BG, Bowler DC, Howland DR (2010) Cough followed by low thoracic hemisection in the cat. Exp Neurol 222:165-170.

Jansen VA, Allain WI, Crane SA (2019) Role of propriospinal neurons in control of respiratory muscles and recovery of breathing following frontotopic injury. Front Syst Neurosci 13:141.
Sherman MF, Lam T, Sheel AW (2009) Locomotor-respiratory synchronization after body weight support treadmill training. J Neurotrauma 26:1191-202.

Nicolae AN, Chui SY, Spence J, Mandel P, Liang J (2016) Experimental modeling of spinal cord injury in adult mice. J Neurotrauma 33:1375-1386.

Streeter KA, Sherman MD, Patel S, Gonzalez-Rothi RJ, Reijerkerk J, Reijerkerk PJ, Dammann K, Robins S, Shi X, Rittschof D (2012) Single-session effects of acute intermittent hypoxia on breathing function after human spinal cord injury. Am J Physiol Regul Integr Comp Physiol 303:R16-28.

Sato H, Arai N, Muto K, Kondo Y, Song H, Nakagawara A (2018) Cervical spinal cord injury: functional rehabilitation in the adult CNS. J Neurol Sci 389:122-129.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

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Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

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Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.

Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG (2018) Cervical spinal cord injury: a state-of-the-art review. Chest 155:438-445.