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Respiratory neuroplasticity and cervical spinal cord injury: translational perspectives

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Abstract

Paralysis of the diaphragm is a severe consequence of cervical spinal cord injury. This condition can be experimentally modeled by lateralized, high cervical lesions that interrupt descending inspiratory drive to the corresponding phrenic nucleus. Although partial recovery of ipsilateral diaphragm function occurs over time, recent findings show persisting chronic deficits in ventilation and phrenic motoneuron activity. Some evidence suggests, however, that spontaneous recovery can be enhanced by modulating neural pathways to phrenic motoneurons via synaptic circuitries which appear more complex than previously envisioned. The present review highlights these and other recent experimental multi-disciplinary findings pertaining to respiratory neuroplasticity in the rat. Translational considerations are also emphasized, with specific attention directed at the clinical and interpretational strengths of different lesion models and outcome measures.

Introduction

Among the more significant advances in spinal cord injury (SCI) research is growing recognition of the capacity for spontaneous recovery [1,2]. Intraspinal neuroplastic reserve in human subjects has been revealed by a range of outcome measures, and improvements can evolve months to years after trauma [3], even in cases of neurologically complete injury [4]. This has led to a greater appreciation for opportunities to therapeutically amplify natural recovery processes, as demonstrated by experimental studies [5,6].

Although most attention to spinal cord neuroplasticity has centered on locomotor function, it is well established that a significant potential for spontaneous recovery also exists in another motor domain referred to here as the phrenic motor system. Approximately half of spinal injuries occur at cervical levels [7], and in those cases involving diaphragm dysfunction, intensive post-SCI care and management are usually required [8–10]. Because such individuals are at risk of increased morbidity and mortality due to secondary pulmonary complications [8,11], continuing development of strategies to improve respiratory function is necessary.

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Respiration after cervical SCI has thus attracted increasing scientific attention largely through integration of SCI and respiratory neurobiology expertise. As with locomotion, current literature suggests that respiratory neuroplasticity might also be responsive to therapeutic intervention [12–14]. In the present review, we first establish a clinical perspective with consideration of respiratory outcome measures, their interpretations and how some approaches might lend to clinical and laboratory application. Discussion then turns to recent experimental findings pertaining to mechanisms and neural substrates associated with phrenic motoneuron (PhMN) function and respiratory behavior after SCI. An additional emphasis of this review is on preclinical modeling considerations that can be pivotal in guiding future research and treatment approaches to optimize post-SCI respiratory neuroplasticity.

Clinical features of respiratory function following spinal cord injury

Respiratory compromise can result from trauma at any spinal segment from high cervical to midlumbar levels owing to impaired primary or accessory respiratory muscle activity [9,11]. A frequently cited example is injury sustained at high cervical levels which can result in diaphragm dysfunction due to interruption of bulbospinal respiratory drive to PhMN pools (C3–C5). In contrast, normal breathing and defensive mechanisms (e.g. cough) can be significantly compromised by more caudal trauma at lower cervical and thoracic levels. This can be attributed to damage affecting descending respiratory fibers to other motoneuron pools (e.g. intercostal and abdominal [15]) or direct damage to motoneurons themselves.

Despite their vulnerability after SCI, the networks controlling various respiratory muscles also possess resiliency. Irrespective of initial deficit, ventilatory improvements are often seen clinically months after injury [16,17]. However, the extent of recovery can vary due to lifestyle and other differences among individuals (e.g. age, history of smoking [16,18]). Spontaneously occurring improvements are often suboptimal, and many individuals who are ventilator weaned continue to experience shortness of breath, weakened phonation and impaired cough. The altered respiratory patterns of such individuals are generally characterized by increased breathing frequency and decreased tidal volume. This most likely reflects an attempt to increase respiratory drive to maintain blood-gas homeostasis. Unfortunately, this rapid shallow breathing pattern can lead to inefficient gas exchange [19], respiratory muscle fatigue [20] and eventually respiratory failure [8].

Although significant improvements in clinical management of respiratory dysfunction (e.g. respiratory muscle pacing [9]) are being made, therapeutic interventions to enhance intrinsic recovery mechanisms might provide a more effective long-term approach. However, the latter will require a better understanding of the interrelationships between different forms of respiratory recovery. For example, pulmonary improvements after SCI are often related to compensatory breathing strategies (e.g. recruitment of accessory respiratory muscles) [21] and altered respiratory muscle biomechanics [17]. Some observations also suggest that changes in PhMN activity might account for slowly evolving diaphragm recovery in some patients [22, 23]. As discussed below, these are not necessarily mutually exclusive phenomena.

Outcome measures of respiratory function and preclinical studies

With expanding interest in applied SCI research, emphasis has been placed on the need for sensitive and quantitative outcome measures to demonstrate the efficacy of novel therapeutic approaches in preclinical investigations and future clinical trials [24]. Although a variety of neurological and functional tests have been discussed [25], little attention has been given to respiratory assessment with translational relevance. Table 1 summarizes available outcome measures with commentary on their individual technical merits and interpretations.

The most frequently used clinical assessment of respiratory dysfunction is spirometry, which measures ventilation under elevated respiratory drive (e.g. forced expiratory volume; see Table 1). Unfortunately, this approach requires subject cooperation and is therefore not feasible in animal studies. Plethysmography is a clinically relevant alternative and has the experimental advantage that even small deficits can be detected by increasing the animal's respiratory drive during brief periods of hypercapnic or hypoxic challenge [26–28].

In addition to plethysmography, assessment of respiratory function in experimental models has focused predominantly on neurophysiological recordings of phrenic nerve activity (also known as phrenic neurograms or compound motor action potentials; CMAP) and muscle electromyography (EMG). Such techniques more accurately detect deficits at identifiable levels of individual respiratory circuits. Recent advances in clinical testing have provided similar methods (e.g. CMAP and EMG) for assessing respiratory circuitry in humans [29–31] (Table 1). Furthermore, some of these tests can predict the long-term functional outcome of injured individuals [31]. However, the information provided by each outcome measure is very different (Table 1) and not always causally related. For example, a change in phrenic nerve or diaphragm muscle activity might not necessarily reflect a change in ventilation, which is a complex respiratory behavior involving the activity of multiple muscle groups (e.g. phrenic, intercostal, abdominal). Likewise, there can be recovery of PhMN activity without a concomitant change in diaphragm [32]. As demonstrated by the recent trend in experimental studies, combined testing approaches provide a more comprehensive and accurate evaluation of respiratory function [26,27,33].

Experimental models of cervical SCI

The term 'neuroplasticity' is not commonly used in the clinical literature in association with recovery of respiratory function after SCI. Therefore, experimental demonstrations of this concept could facilitate future therapeutic developments. Essential to this are preclinical models approximating the human condition or capable of providing fundamental proof-of-principle information. Of all experimental cervical spinal lesion paradigms used to assess respiratory function after SCI (Figure 1; Table 2), the 'crossed phrenic phenomenon' (CPP) [12] has provided the most evidence for enduring functional changes with associated synaptic remodeling after injury. In this model, *complete* lateral hemisection (HMx) at the second cervical segment (i.e. C2) interrupts descending inspiratory drive on one side of the spinal cord and results in ipsilateral diaphragm paralysis (Figure 1a). Although it has been reported that incomplete lateral hemisection (with some medial white matter sparing) results in a similar functional outcome [32,34], as these prior reports acknowledged, further elucidation of the underlying anatomy is required. Although some studies have noted the presence of bulbospinal-phrenic pathways in the ventromedial white matter [35,36], it is possible that these fibers do not subserve PhMN function [32]. Phrenic neurograms and diaphragm EMG (Table 1) have shown reactivation of ipsilateral PhMNs, either spontaneously following lateral C2HMx [26, 27,37–40] or as a consequence of subsequent contralateral phrenicotomy [12,41]. Recovery has been attributed to activation of a preexisting latent pathway originating from intact contralateral bulbospinal axons that cross at the level of the phrenic nucleus and terminate monosynaptically on ipsilateral PhMNs (i.e. the CPP). Synaptic remodeling occurs in the phrenic nucleus after C2HMx and appears to precede expression of the CPP (reviewed in Ref. [12]). As indicated in Table 2, the CPP has been demonstrated in many species. This includes recent demonstration of the CPP in mice [42,43], which opens the possibility for genetically probing mechanisms of respiratory neuroplasticity.

An acknowledged translational shortcoming of the CPP model is that laceration-type injuries analogous to C2HMx are rare in humans. Spinal contusions instead represent the most frequent lesion type and present different pathophysiological and histological conditions. Following on

an earlier study of cervical contusion injuries [44], Baussart *et al.* [45] recently examined respiratory consequences of lateralized C2 contusions (Figure 1b). As in the case of C2HMx, severe lateral contusions will interrupt respiratory drive to PhMNs. However, any white matter sparing (dependent on injury severity) is a critical interpretational consideration owing to lateral and ventromedial distribution of bulbospinal respiratory fibers [36] and the different functional outcomes [44,45] (see Figure 1b,v).

The lateralized C2 contusion approach is an important complement to the C2HMx model, and clinical reports of such injuries have been made [46]. The practical relevance of such lesions, however, still appears modest, as most injuries of that nature in the human are near midline and often exhibit only moderate asymmetry (Figure 1d). In addition, there is a propensity for contusions to occur at midcervical levels (e.g. [47]). Midcervical contusion injury models have been extensively characterized [48–50], with some reports addressing respiration after either lateralized or midline C4–C5 contusions [28,44] (Figure 1c,d). However, the pathological features of these lesions differ strikingly from conditions associated with high cervical injuries as discussed below. The C2HMx model is nevertheless a more consistent lesion affecting an identified population of descending respiratory axons. As such, the CPP provides a fundamental example of respiratory neuroplasticity in which the underlying circuitry can be more clearly defined and its functional role in recovery analyzed. These considerations lead to an important question, namely, to what extent does the CPP account for any improvements in breathing?

Patterns of breathing after cervical SCI

Previous work suggests the CPP has little impact on breathing behavior during the first 5 weeks post-C2HMx [27]. Plethysmography studies of unanesthetized rats demonstrated that minute ventilation was maintained but with a rapid, shallow breathing pattern under room air (i.e. ‘quiet breathing’) conditions, similar to what is seen in humans (see above). Moreover, persistent deficits in ventilation were observed during conditions of increased respiratory drive, and parallel studies in anesthetized rats indicated minimal recovery of ipsilateral phrenic output (i.e. the CPP) [27].

A more recent investigation extending assessment to 12 weeks post-C2HMx showed gradual, but continual, improvement in ventilation during baseline and hypercapnic conditions [26]. These findings might reflect increased reliance on the CPP, but the nature of these outcome measures (specific neurophysiological activity versus gross respiratory behavior) does not permit direct correlation between results, for reasons noted earlier (see also Table 1). Indeed, the observation that ipsilateral phrenic activity recovers after C2HMx, but before ventilatory improvement is observed, brings into question the contribution of the CPP to overall respiratory outcome. However, Golder *et al.* [51] demonstrated that activation of ipsilateral PhMN pool after C2HMx does contribute to ventilation under certain conditions. Specifically, there was a significant reduction in the volume of augmented breaths (i.e. ‘sighs’) and decreased tidal volume when recovered PhMN activity was prevented from reaching the diaphragm by an ipsilateral phrenicotomy. The augmented breaths described require elevated neural drive and increased PhMN recruitment [52,53]. The functional outcome of the CPP might thus only be detectable under conditions of respiratory challenge and greater respiratory drive. Collectively, existing data suggest that neuroplasticity associated with the CPP might contribute to respiratory recovery after C2HMx, but this mechanism remains insufficient to promote full respiratory recovery.

More recently, Choi *et al.* [28] examined breathing patterns following lateralized C5 contusions of graded severity. That study showed that, under room air conditions, the injured rats had reduced tidal volumes (V_t) and increased respiratory frequency (f) during the first 2 weeks post-

SCI, and the magnitude of these changes was positively correlated with injury severity. Thereafter, however, rapid shallow breathing transitioned to a normal respiratory pattern. Alterations in breathing during hypercapnic respiratory challenge (e.g. reduced V_t and f) were more persistent but, by 6 weeks post-injury, breathing in contused rats became comparable to controls under these conditions [28]. This more prolonged deficit correlated with global motoneuron loss at the lesion epicenter. Therefore, respiratory outcome measures following midcervical contusion in rats are likely to be at least partially determined by the relative degree of PhMN loss, as has been suggested in humans [30].

Spontaneously improved diaphragm function is likely to be an important part of the recovery process, but in what context *neuroplasticity* might play a role in midcervical injury models and humans is unclear. First, given midline tissue damage even with a lateralized contusion, a CPP-like contribution might be impaired, resulting from damage to the crossed 'CPP pathway' (Figure 1c,d). Second, midcervical contusions will also entail variable white matter damage on both sides (e.g. [50]). Third, some uni- or bilateral loss of PhMNs will occur which has been correlated with ventilatory function [54]. How such relative disruption of white or gray matter affects respiratory neuroplasticity following contusive injury is yet to be defined. It is thus likely that plasticity in the phrenic motor system can be differentially expressed depending upon the segmental level and severity of the injury. Whereas severe midcervical contusion injuries would presumably show greater respiratory dysfunction, such trauma in rodents (i.e. approximating a ventilator-dependent human) is not immediately feasible [28]. The preclinical value of experimental midcervical injuries must therefore be gauged by what clinically relevant features can be defined. For example, it is conceivable that deficits observed during conditions of increased respiratory drive in animals might reflect what is experienced by a ventilator-weaned individual whose overall respiratory capacity is still weak. By contrast, any change in diaphragm function seen experimentally and clinically might reflect neuroplasticity via mechanisms other than the CPP. This might entail reorganization of neuromuscular junctions at the level of the diaphragm [55,56], altered recruitment of remaining PhMNs [55], intrinsic changes in PhMNs themselves [33,57] or changes in pre-phrenic circuitries above or below the injury site, among other possibilities. In addition, some functional recovery observed following midcervical SCI might be attributed to compensatory mechanisms such as recruitment of alternative respiratory pathways unaffected by injury. Such changes have been observed with SCI at other vertebral [58] and brainstem [40] levels, and might represent another expression of respiratory plasticity [40]. For example, Golder *et al.* [40] demonstrated that C2HMx in the rat also resulted in concomitant changes in hypoglossal nerve activity. The overall pattern of ventilation observed following cervical SCI could thus be a result of the combined effects of neuroplasticity within the phrenic circuit and compensatory recruitment of unaffected, accessory respiratory circuits.

Finally, although the focus of the present review is predominantly on descending respiratory drive, one consideration is that spinal cord injury might also disrupt afferent input to spinal respiratory cells. Furthermore, several studies have shown that both ipsilateral and contralateral afferent input to phrenic neurons can influence expression of the CPP [34,59]. Goshgarian [59] demonstrated that acute contralateral rhizotomy following a lateral C2HMx elicited an earlier and more robust activation of the ipsilateral phrenic motor pool than would be seen via the spontaneous CPP alone. However, the underlying mechanisms and neural circuitries are poorly understood.

Enhancement of respiratory recovery

Whereas the clinical approach to respiratory dysfunction post-SCI has primarily focused on management and assisted ventilation, other approaches have been employed to enhance post-SCI pulmonary function such as respiratory muscle strengthening (reviewed in Ref. [60]). In

addition, experimental evidence has shown that ipsilateral phrenic nerve activity post-C2HMx can be significantly enhanced by surgical [59,61] or physiological [37,39] approaches. Such findings suggest that chronic respiratory deficiency might not be an insurmountable condition. This view has been more firmly anchored by studies showing that a variety of neurotransmitters/neuromodulators (Table 3) can enhance neuroplasticity in the phrenic motor system. For instance, it has been shown that increased expression or activation of serotonin and 5HT receptors might mediate the onset and extent of respiratory plasticity post-injury [28,54,62–64]. Contralateral cervical rhizotomies, as discussed above, also increase serotonergic input onto PhMNs [65]. Pharmacological studies have revealed a likely role for glutamatergic receptors in mediating plasticity post-C2HMx [43,66]. These alterations in neurotransmitter/receptor expression enhance motoneuron excitability [67]. Recent studies have shown a role of adenosine receptors in respiratory plasticity post-SCI, with particular focus on pharmacological manipulation of receptor activity [33,68,69]. Activation of the central adenosine receptor A2a induces a persistent phrenic motor facilitation and enhances synaptic strengthening at the spinal level in uninjured animals [33]. Furthermore, administration of adenosine agonists following C2HMx significantly increases tidal volume [33].

Respiratory neuroplasticity post-SCI also appears to involve molecular changes downstream to activated neurotransmitter receptors by mechanisms (e.g. involvement of trophic factors [57]) similar to what has been described in other cellular models of neuroplasticity. It has become evident that expression and regulation of neuromodulators and neurotrophins are intimately related to each other. Recently, Baker-Herman *et al.* [70] showed serotonin-dependent BDNF synthesis in cervical ventral gray matter containing the phrenic nucleus that was associated with intermittent hypoxia and long-term facilitation.

Finally, inhibitory neurotransmitters have been implicated in mediating plasticity post-SCI [71]. Inhibition of ipsilateral PhMN activity by contralateral afferents has been demonstrated in several animal models, attributed to γ-aminobutyric acid (GABA) [71]. Therefore, the onset and extent of recovery associated with plasticity post-SCI represents a balance between mechanisms that can both inhibit and excite respiratory motor output.

Collectively, these findings reveal several potential targets for promoting plasticity, and some treatment strategies exploiting neuromodulatory actions post-injury have been explored (e.g. plasminogen activator [43]). In addition, there has been growing interest in strategies designed to promote axonal growth following injury that might also enhance respiratory recovery post-SCI (e.g. cell/tissue transplants [72,73], increased cAMP [74]) or attenuate molecules inhibitory to axonal growth (e.g. chondroitin sulfate proteoglycans [75]).

Neural circuitry and respiratory recovery

To date, enhanced respiratory-related synaptic plasticity has been attributed to changes primarily at the level of the PhMN, as exemplified by neurophysiological studies [37,76], Some synaptic plasticity might also entail respiratory-associated changes elsewhere in the cervical spinal cord, as well as at brainstem levels. Present neuroanatomical data indicate that the crossed axons mediating the CPP derive from decussating contralateral and recrossed ipsilateral axons from the ventral respiratory column (VRC) [77,78]. Otherwise, little is known about changes in descending inputs to PhMNs from other regions of the medulla following cervical spinal cord injury. Within the spinal cord, phrenic neurograms post-C2HMx have demonstrated a delayed, rather than the normally synchronous, onset of recovered ipsilateral PhMN bursting relative to contralateral PhMN output [26]. This raises the possibility that PhMN activity after C2HMx might be affected by a neural substratum that *is not exclusively limited* to intact monosynaptic projections from the VRC. Whereas anatomical studies have

demonstrated the presence of inter-neurons associated with the phrenic circuit of rat [79] and ferret [80], they have been largely dismissed in the rat as having an insignificant role in respiration. However, given their connectivity and potential for modulating PhMN activity, these cells might assume greater functional significance after SCI.

Polysynaptic inputs to PhMNs have been demonstrated by neurophysiological studies in different species [81–83]. Although the existence of propriospinal relays between the medulla and phrenic motoneurons in the rat was previously rejected based on negative neuroanatomical tracing results [77,84], recent observations provide an alternative perspective [85]. Delivery of pseudorabies virus (PRV) to one-half of the diaphragm revealed a substantial population of pre-phrenic interneurons distributed bilaterally throughout the cervical spinal cord in the dorsal horn and around the central canal. When combined with a physiologically targeted anterograde tracing approach, labeled VRC terminals were observed in close proximity to primary dendrites or cell soma of some of these pre-phrenic interneurons [85] (see also Figure 2). Although their role is currently unknown, there is some neurophysiological evidence that activation of polysynaptic spinal pathways can contribute to enhanced phrenic output after C2HMx [76]. Thus, recovery of PhMN activity in the C2HMx model might act in part via spinal interneurons, and this raises consideration for the functional recruitment of preexisting ‘bypass’ pathways following injury, as has been described in other SCI models [86,87].

There is, therefore, an increasing body of evidence demonstrating that respiratory circuits are modulated by other cells within the spinal cord. Evidence for interneurons contributing to alternative pathways and locomotor neuroplasticity post-SCI has been derived from hemisection lesion models. However, whether these cells play a significant role in various forms of functional recovery after contusion injury has not been determined. In terms of respiratory neuroplasticity, interneurons might provide an alternative substrate to either maintain connectivity with spared PhMNs or form novel functional circuits with accessory respiratory motoneuron pools (e.g. spared intercostal motoneurons). Then again, some interneurons might attenuate the extent of recovery if they are inhibitory. A future challenge is to obtain greater understanding of the functional connectivity of these cells to better define sites of action of various therapeutic approaches which can be complementary to respiratory rehabilitation or other strategies.

Closing commentary: translational relevance of respiratory neuroplasticity models

Recent animal data, based upon the CPP model, continue to highlight the possibility that enhancement of phrenic neuroplasticity represents an achievable therapeutic target in SCI research. However, the pathology and cervical level of human contusions are often inconsistent with preservation of the neural substrate mediating the CPP (Figure 1). Therefore, from a clinical perspective, respiratory neuroplasticity as defined by the CPP might represent a biological phenomenon unique to a particular lesion model. To address this and related issues, this discussion has considered some of the relative translational and theoretical merits of the CPP and other cervical SCI models of respiratory dysfunction.

The preclinical value of experimental models would be considerably enhanced by more comprehensive documentation of the respiratory consequences following human SCI, with greater attention to lesion imaging and neurophysiological changes. In that respect, translation is a bidirectional process, and the significance of animal data is dependent on clinical observations that can influence experimental designs and interpretations. One consideration is that diaphragm dysfunction is a probable consequence of high-to-midcervical SCI in humans, irrespective of injury laterality. Although lesion anatomy in humans is highly variable, the functional outcomes observed both clinically and experimentally are relatively comparable

depending on the outcome measures employed. In that regard, despite its relatively unique features, the CPP has provided an appreciation of spinal cord neuroplasticity that can be of significant clinical value.

One of several future challenges will be to determine how neuroplasticity relates to respiratory improvements following midcervical contusions. An experimental contusion severe enough to completely replicate a ventilator-dependent human condition is unlikely to evolve. Apart from the logistical and ethical considerations of restricting a conscious animal to mechanical ventilation, studies have shown that diaphragm atrophy occurs within hours of mechanical ventilation [88]. It therefore becomes important to identify how functional parameters of less damaging injuries relate clinically.

As noted, outcome measures exist that can be applied both in humans and animals which can greatly enhance translational power. Studies to date indicate similarities in breathing patterns seen after midcervical contusions in humans and rats. To what extent progressive spontaneous improvements in ventilation entail another form of neuroplasticity after injuries involving gray and/or white matter components remains to be determined by further functional and correlative neuroanatomical investigations.

In principle, each lesion type addressed in this review has translational strengths depending on the issues addressed and evolving concepts. In that regard, the hemisection and contusion models studied can both be instrumental to discovery of pharmacological targets and related molecular mechanisms that can increase the synaptic efficacy of the denervated circuit (i.e. pre- or postsynaptic plasticity) or augment intrinsic neuronal excitability [28,33,62,66,68,71]. Recognition of neuroplasticity as a basis for respiratory recoveries in some patients could influence a paradigm shift in rehabilitation and other treatment approaches to reverse a potentially life-threatening consequence of cervical SCI.

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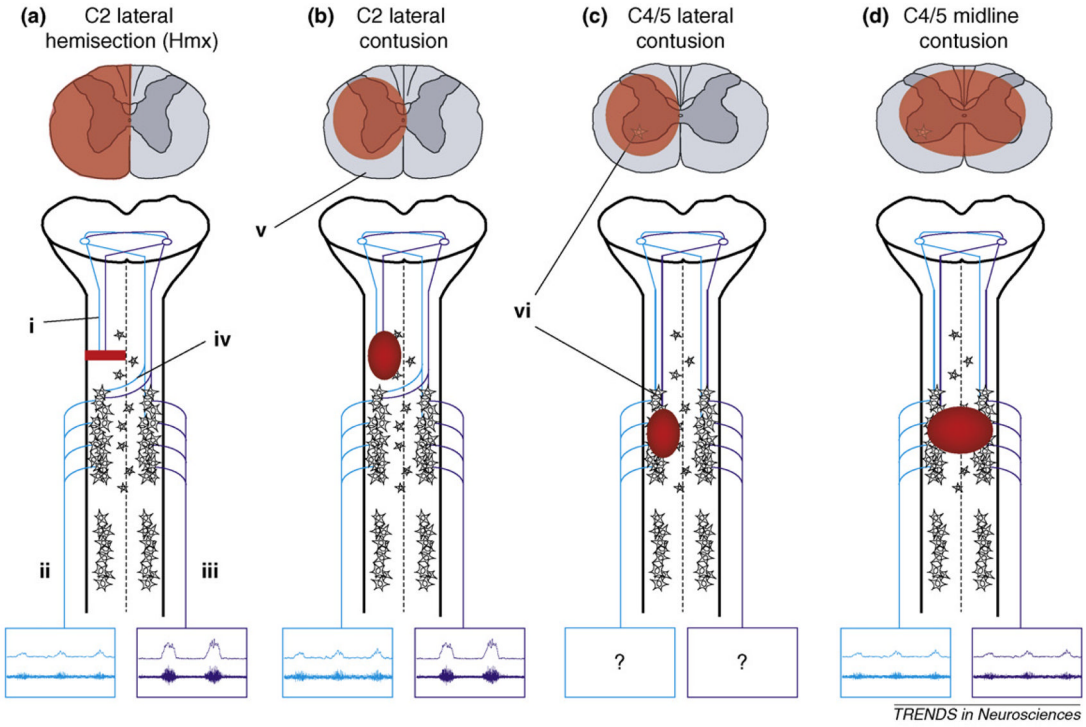

Figure 1.

Schematic diagrams outlining cervical injury models that have been used to examine respiratory function. Diagrams of spinal cord cross-sections demonstrate general morphological features (highlighted in red) of each injury type. Emphasis is given to descending pathways from the medulla (i) to PhMNs, which control diaphragm function via the phrenic nerve. Examples are presented of how each injury type affects efferent burst activity (both raw [top] and integrated [bottom] neurograms) recorded in the phrenic nerves (ii, iii) on each side of the spinal cord. The C2HMx **(a)** is the most frequently examined model and has provided the best evidence for respiratory neuroplasticity post-SCI. Following interruption of ipsilateral input from the medulla, spontaneous recovery of ipsilateral phrenic nerve activity (ii) has been observed. This recovery, known as the crossed phrenic phenomenon (CPP), is attributed to latent pathways (iv) that cross the spinal midline at the level of PhMNs. Note that following lateral injuries (a,b), in addition to ipsilateral recovery, contralateral phrenic activity is increased relative to normal. As contusive injury occurs more frequently in human SCI, some studies have begun to compare lateral C2 contusions **(b)** to the C2HMx model. However, depending on the extent of injury, contusive lesions usually result in some degree of white matter sparing (v). Therefore, in addition to the crossed, contralateral pathways of the CPP, some ipsilateral input from the medulla might be spared. As the majority of human SCIs occur in the midcervical region (C4/5), some studies have also begun to address the effects of such injuries on respiration **(c**, **d)**. How lateral injuries at this level affect phrenic output is yet to be determined. However, as indicated, lesions at the midcervical levels also result in loss of PhMNs (vi). Furthermore, lesions that extend toward the spinal midline (d) are likely to interrupt crossing pathways associated with the CPP.

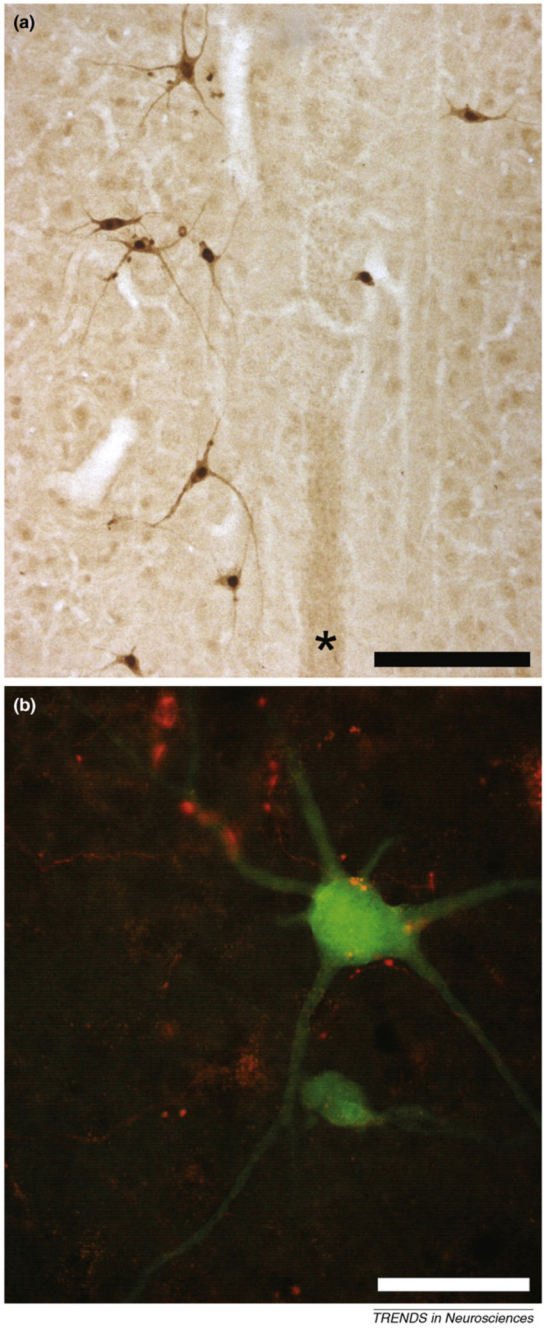

Figure 2.

Photomicrographs of longitudinal sections (40 microns) from the cervical spinal cord of an adult rat. Within 48 h following delivery of the transsynaptic tracer pseudorabies virus (PRV) to the left hemidiaphragm, the ipsilateral phrenic motoneuron pool becomes infected with the retrogradely transported virus. Subsequently (64 h; [a,b]), pre-phrenic interneurons become transsynaptically infected. **(a)** Antibodies against PRV were used to immunocytochemically visualize PRV-infected cells at around laminae VII/X. These interneurons are bilaterally distributed around the central canal and spinal cord midline (*). **(b)** This pre-phrenic interneuron (green) has been retrogradely infected with the PRV that results in expression of green fluorescent protein. Projections from the ventral respiratory column in this animal were

anterogradely labeled using MiniRuby (red). These projections appear in close contact with the soma and dendrites of the interneuron, which could suggest synaptic contact. This finding (described in detail by Lane *et al.* [85]) indicates that whereas there is substantial monosynaptic bulbospinal-phrenic projection, there is also anatomical evidence for a polysynaptic pathway in the phrenic circuit of the rat. This pathway might serve as an alternative to direct monosynaptic projections from the medulla following spinal cord injury and represent a viable therapeutic target. Scale bar is 200 (a) and 50 (b) micrometers.

Table 1

Clinical and experimental measures of respiratory function

Blood gases

Use: clinical and some experimental	Considerations
- Measure of oxygen and carbon dioxide partial pressures, and pH from blood sample	- Repeated measures in animals can be limited by the small blood volume of most experimental animals
- As such, it is the most accurate reflection of respiratory efficiency (adequacy of gas exchange)	- In many cases, sampling from awake animals is difficult without causing stress (directly affecting respiration)
- Blood samples have potential for used in assessing biomarkers of injury, plasticity and repair	- Difficult to relate to underlying anatomy and might be influenced by circulatory dysfunction

Oximetry

Use: experimental and clinical	Considerations
- Measure of hemoglobin saturation in arterial blood	- Oxygen saturation might not be compromised in common experimental SCI models, or might only be reduced following severe injury
- Noninvasive	- Cannot determine concentration of carbon dioxide or sodium bicarbonate, or pH
	- Might not directly correlate with ventilation

Spirometry

Use: clinical	Considerations
- Uses a pneumotachograph to measure airflow to and from lungs	- Might reflect compensatory mechanisms by unaffected circuits (e.g. increased intercostal activity might offset diaphragm dysfunction, so parameters of ventilation are relatively unaffected). Thus, this method does not necessarily provide information about mechanisms of recovery. However, as respiration can be measured in absolute units, we can determine with some certainty whether the overall output of the respiratory system is altered by injury or a particular intervention.
- Routinely used clinically as a pulmonary function test to determine multiple parameters, including forced vital capacity (FVC), forced expiratory volume per second ($FEV_{1.0}$), inspiratory capacity (IC) and peak expiratory flow (PEF)	- Requires voluntary control of respiration and therefore cooperation and motivation of the subject
- Noninvasive	
- The 'sniff' test can be used as an additional measure for assessment of diaphragm function under increased drive	

Plethysmography

Use: experimental and clinical	Considerations
- Measure of ventilation (gross measure of respiratory behavior) in terms of breathing frequency (f), tidal volume (V_T) and minute ventilation (V_E)	- Can reflect compensatory mechanisms by unaffected circuits as described for spirometry
- Noninvasive	- Measure is minute ventilation and not alveolar ventilation. Thus, changes in pulmonary dead space could lead to misleading conclusions.
- Clinically, can determine functional residual capacity more accurately than spirometry	- Common interferences to ventilation need to be carefully controlled

Imaging techniques (including chest X-ray, CT, ultrasound, fluoroscopy); see Ref. [89] **for discussion**

Use: experimental and in some clinical circumstances	Considerations
- Visual representation of dysfunction (chest X-ray and CT)	- Usually only suggestive of dysfunction and requires confirmation with additional outcome measures
- Ultrasonography assesses real-time movement of diaphragm	
- Can be used to determine diaphragm thickness and change in muscle thickness during respiratory activity	

Electromyography (EMG)

Use: experimental and in some clinical circumstances	Considerations
- Measure of muscle activity	- Muscle activity does not necessarily reflect a behavioral effect
- Chronic placement of electrodes can be used for repeated measures	- EMG recordings cannot sample the activity of the entire pool of active motor neurons. Because there are regional differences in the activation of respiratory muscles (e.g. costal versus crural diaphragm), recording from a single site might not accurately reflect diaphragm function.
- Can be noninvasive depending on muscle of interest	- Muscle atrophy could alter the signal
	- Clinical recording is usually made noninvasively, placing electrodes on skin surface, decreasing accuracy of recording

Nerve conduction recording using diaphragm CMAP (compound motor action potential)

Use: Clinical	Considerations
- Noninvasive (electrode placed on skin)	- Electrode placement might not detect solely the nerve of interest
- *Indirect* measure of nerve function by assessing nerve integrity	- High-intensity stimulus is required, which might cause discomfort to subject
- Uses either electrical or magnetic stimulation	

Direct neurophysiological recording (exemplified by nerve recording described here)

Use: Experimental (animal models) only	Considerations
- Direct measure of nerve function and relative motoneuron output	- Most neurophysiological recordings (e.g. from phrenic nerve) are terminal experiments
- The amplitude of the nerve burst reflects:	- Nerve activity does not necessarily reflect muscle activity
(i) the number of active motoneurons	- Nerve output does not necessarily reflect behavior, particularly because most studies are done under anaesthesia
(ii) the frequency of motoneuron discharge	- The amplitude of the nerve burst is also dependent on:
- Can compare between output from nerves on each side	(iii) electrode placement (proximity of the electrode to axons of active motoneurons)
- In the standard preparation (e.g. anesthetized, vagotomized, ventilated, etc.), many factors influencing breathing can be limited, and thus the output of a particular motor pool (e.g. phrenic) can be studied under carefully controlled conditions	(iv) the configuration of the recording system
- Can directly assess effect of treatment (serotonin agonists) and/or challenge (hypoxia) on motoneuron output	- Quantifying nerve output is problematic and there is no universally accepted method for quantifying the burst amplitude

- The peak height of the integrated phrenic inspiratory burst is highly correlated with peak airway pressure. Accordingly, many researchers have used this as an index of 'respiratory neural drive.'	- Comparing the burst amplitude between two nerves in the same rat (e.g. IL versus CL) or across rats can be problematic(e.g. even output in uninjured animals can differ between sides) - Normalizing phrenic bursting to a maximum (e.g. asphyxic bursting) can remove physiologically meaningful differences between groups, as maximum bursting is reduced after SCI

Summary of some of the outcome measures used to assess respiratory function experimentally and/or clinically. The extent and detail of information provided by each test and necessary considerations are addressed. The most effective assessment of respiratory function is likely to come from use of multiple functional tests (e.g. nerve recording to assess function at the circuitry level, relative muscle activity assessed with EMG, plethysmography to measure overall breathing patterns and blood-gas analysis to determine respiratory efficiency).

Table 2

Experimental models of SCI used to assess respiratory dysfunction

Spinal level	Injury model	Recovery	Species
C1	Complete transection	Spontaneous (but temporary)	Dog [90,91], cat [92], rat [93]
C2	Complete transection	Spontaneous (but temporary)	Dog [90,91], rabbit [94]
	Lateral hemisection	CPP: induced by contralateral rhizotomy	Dog [41,95], cat [95], rabbit [41,95], rat [96], mouse [42,43], woodchuck [95], guinea pig [58]
	Lateral hemisection	CPP: spontaneous	Cat [97], rabbit [97], rat [26,27,37–40]
	Incomplete lateral section	Spontaneous phrenic nerve activity, but no diaphragm EMG recovery	Rat [32,34]
	Complete transection	Induced by anastomosis and innervation by spared systems	Rat [98]
	Lateral hemicontusion	Spontaneous recovery	Rat [44]
	Lateral hemicontusion	No recovery	Rat [45]
C4/5	Midline contusion	Spontaneous recovery	Rat [44]
C5	Lateral hemicontusion	Spontaneous recovery, but enhanced via serotonergic agonists	Rat [28]
T8	Midline contusion	Induced by administration of serotonergic agonists	Rat [54,64]

This table summarizes some of the injury models that have been used to examine respiratory dysfunction, with reference to potential mechanisms of recovery and the animals used. Note that these examples reflect studies in both male and female animals, at a range of weights and ages. The C2 lateral hemisection (C2HMx) is the most extensively documented model and has provided the most comprehensive evidence for neuroplasticity in the injured respiratory circuitry.

Table 3

Pharmacological mediators of respiratory plasticity post-SCI

Molecule	Receptor	Expression post-injury	Anatomical location of expression	Effect on plasticity
Serotonin	5HT-1A	Increased	Dorsal horn [62,64] PhMN [64]	Enhancing
	5HT-2A	Increased	Medulla [99]	Enhancing
			Spinal cord [99], PhMN [63]	Enhancing
Glutamate	AMPA-GluR1	Increased [66]	PhMN [66]	Enhancing
	AMPA-GluR2	Decreased [66]	PhMN [66]	Enhancing
	NMDA–2A	Increased [66]	PhMN [66]	Enhancing
GABA	GABA-A	—	Dorsal horn [71]	Inhibiting
	GABA-B	—		Inhibiting
Adenosine	Adenosine–A1	—	Carotid bodies [69]	Inhibiting
	Adenosine–A2a	—	Carotid bodies [69] PhMN [33]	Enhancing

Summary of some of the reported molecules and receptors that can influence the onset and extent of respiratory plasticity following spinal cord injury. Identification of these neuromodulators, and an increasing interest in their role following injury, has led to the development of several therapeutic approaches to improving respiratory function post-SCI.