Recovery of the pulmonary chemoreflex and functional role of bronchopulmonary C-fibers following chronic cervical spinal cord injury

Kun-Ze Lee1,2,3,4 and Yu-Shuo Chang1

1Department of Biological Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan; 2Institute of Medical Science and Technology, National Sun Yat-sen University, Kaohsiung, Taiwan; 3Center for Neuroscience, National Sun Yat-sen University, Kaohsiung, Taiwan; and 4Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung, Taiwan

Submitted 11 August 2014; accepted in final form 16 September 2014

Lee KZ, Chang YS. Recovery of the pulmonary chemoreflex and functional role of bronchopulmonary C-fibers following chronic cervical spinal cord injury. J Appl Physiol 117: 1188–1198, 2014. First published September 25, 2014; doi:10.1152/japplphysiol.00723.2014.—Persistent impairment of pulmonary defense reflexes is a critical factor contributing to pulmonary complications following cervical spinal cord injury. The rapid shallow breathing of C2Hx animals persisted even when the pulmonary chemoreflex has been reported to be abolished in animals with acute cervical hemisection (C2Hx). The present study examined whether the pulmonary chemoreflex can recover during the chronic injury phase and investigated the role of bronchopulmonary C-fibers on the altered breathing pattern after C2Hx. In the first protocol, bronchopulmonary C-fibers were excited by intrajugular capsaicin administration in uninjured and complete C2Hx animals 8 wk postsurgery. Capsaicin evoked pulmonary chemoreflexes in both groups, but the reflex intensity was significantly weaker in C2Hx animals. To examine whether spared spinal white matter tissue contributes to pulmonary chemoreflex recovery, the reflex was evaluated in animals with different extents of lateral injury. Linear regression analyses revealed that tidal volume significantly correlated with the extent of spared tissue; however, capsaicin-induced apnea was not related to injury severity when the ipsilateral-to-contralateral white matter ratio was <50%. In the second protocol, the influence of background bronchopulmonary C-fiber activity on respiration was investigated by blocking C-fiber conduction via perivagal capsaicin treatment. The rapid shallow breathing of C2Hx animals persisted after perivagal capsaicin treatment despite attenuation of pulmonary chemoreflexes. These results indicate that the pulmonary chemoreflex can recover to some extent following spinal injury, but remains attenuated even when there is moderate spinal tissue sparing, and that altered breathing pattern of C2Hx animals cannot be attributed to endogenous activation of bronchopulmonary C-fibers.

pulmonary chemoreflex; cervical spinal cord injury; bronchopulmonary C-fiber; breathing pattern

Address for reprint requests and other correspondence: K.-Z. Lee, Dept. of Biological Sciences, College of Science, National Sun Yat-sen Univ., Kaohsiung, Taiwan (e-mail: kzlee@mail.nsysu.edu.tw).
It is unknown whether expression of the pulmonary chemoreflex following cervical spinal cord injury depends on the extent of spared tissue. Thus our second aim was to evaluate the relationship between the intensity of the pulmonary chemoreflex and the amount of spared white matter tissues in C2Hx animals.

Bronchopulmonary C-fibers, the main chemosensitive vagal afferents in the lung, can detect a variety of internal inflammatory mediators (e.g., cytokine and reactive oxygen species) and inhaled irritants (e.g., smoke and acrolein) (27, 33, 38, 56). Although they usually have low baseline activity (19, 39), bronchopulmonary C-fibers have a background effect on the eugonic breathing pattern (46, 49). Specifically, blockade of vagal C-fiber activity was shown to reduce respiratory frequency and enhance the tidal volume in anesthetized dogs (50), suggesting that background vagal C-fiber activity may trigger rapid shallow breathing. This pattern of breathing has been described during ozone inhalation and experimentally induced pulmonary air embolism and results from activation of bronchopulmonary C-fibers during the resting breathing (6, 49).

In light of these prior findings, we hypothesized that the rapid shallow breathing pattern observed in C2Hx animals may be, at least in part, due to an elevation of background bronchopulmonary C-fiber activity. Therefore, the third aim of this study was to investigate whether the rapid shallow breathing pattern exhibited by C2Hx animals can be reversed by a blockade of background bronchopulmonary C-fiber activity.

MATERIALS AND METHODS

Animals. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the National Sun Yat-sen University. A total of 67 male adult Sprague-Dawley rats obtained from BioLasco Taiwan were studied. Animals were assigned to the uninjured (C2 laminectomy only) \((n = 23)\), complete C2Hx \((n = 15)\), and incomplete C2Hx \((n = 29)\) groups.

Spinal cord injury. At 9 wk of age, all animals were anesthetized with xylazine \((10 \text{ mg/kg sc, Rompun, Bayer, Germany})\) and ketamine \((140 \text{ mg/kg ip, Ketalar, Pfizer})\) and received a dorsal cervical incision from C1 to C3 spinal cord followed by C2 laminectomy after absence of the toe-pitch withdrawal reflex. The left C2 spinal cord was incised by the microscalpel and a lesion cavity was created by the gentle aspiration using micropipette connected to a suction pump in C2Hx group animals. The dura and overlying muscles were closed with 10-0 nylon (UNIK) and 4-0 chronic (UNIK) sutures, respectively. The skin was then sutured with 4-0 nylon sutures (UNIK). Upon the completion of surgical procedures, animals were given subcutaneous injections of yohimbine \((1.2 \text{ mg/kg, Tocris})\) to reverse the action of xylazine, lactated Ringer’s solution \((5 \text{ ml, Nang Kuang Pharmacetical})\) to prevent dehydration, and buprenorphine \((0.03 \text{ mg/kg, Shinlin Sinseng Pharmaceutical})\) for analgesia. The daily oral supply of Nutri-cal \((1–3 \text{ ml, EVSCO Pharmaceuticals})\) and lactated Ringer’s solution injection \((5 \text{ ml sc})\) were applied until adequate volitional eating and drinking resumed.

Animal preparation. Eight weeks after laminectomy or C2Hx surgery, animals were anesthetized with urethane \((1.6 \text{ g/kg ip, Sigma})\) and maintained in a supine position. An adequate anesthesia was confirmed by an absence of the toe-pinch withdrawal reflex. The rats’ rectal temperature was monitored by an electrical thermometer and maintained at \(37 \pm 1^\circ \text{C}\) by placement of the animals on a servocontrolled heating pad (model TC-1000, CWE). The rats were tracheotomized and an endotracheal tube \((\text{PE-240, Clay Adams})\) was connected to a respiratory flow head (MLT1L, ADInstruments) coupled with a spirometer (FE141, ADInstruments) for respiratory flow measurement. The right jugular vein and left femoral artery were catheterized for capsaicin administration and blood pressure measurement (Transducer: DTX-1; Amplifier: BPM-832, CWE), respectively. A hyperoxic gas mixture \((50 \% \text{ O}_2, \text{ balance N}_2\); flow rate: 2 liters/min) was delivered to the endotracheal tube via a “T-piece” connector (9).

All physiological signals (e.g., airflow and blood pressure) were digitized using the CED Power 1401 (Cambridge Electronic Design) and the PowerLab data-acquisition system (ADInstruments). All data were recorded in a computer by the Spike 2 and LabChart software.

Experimental protocol. Two experimental protocols were used to investigate the functional role of the bronchopulmonary C-fiber on breathing following C2Hx. In the first protocol, bronchopulmonary C-fibers were excited by intrajugular administration of capsaicin, which has been widely used to evoke the pulmonary chemoreflex (28–30, 37, 52). The stock solution of capsaicin \((0.5 \text{ mg/ml})\) was prepared in 80% saline, 10% ethanol \((95\%),\) and 10% polysorbate 80, and stored at \(-20^\circ \text{C}\). On the day of the experiment, the stock solution was diluted to the desired concentration with saline based on the weight of the animal. The pulmonary chemoreflex evoked by three doses of capsaicin injection \((0.5, 1.0, \text{ and } 1.5 \mu \text{g/kg})\) was evaluated in uninjured \((n = 12),\) complete C2Hx \((n = 7),\) and incomplete \((n = 29)\) C2Hx animals. Three doses of capsaicin were randomly delivered and an interval of 20 min was allowed between two subsequently injections to prevent tachyphylaxis.

In the second protocol, which was used to investigate the influence of background vagal C-fiber activity on breathing, vagal C-fiber conduction was blocked by perivagal capsaicin treatment \((39, 47–48)\) in both uninjured control \((n = 11)\) and complete C2Hx \((n = 8)\) group. The cervical vagus nerves were exposed bilaterally, separated, and placed on a paraffin. An \(~2 \text{ mm} \) cotton strip presoaked in the capsaicin solution \((0.25 \text{ mg/ml})\) was used to wrap the vagus nerves for 20 min. The vagus nerves were washed with saline after removal of the cotton strip. To evaluate whether vagal C-fiber conduction was blocked by the treatment, a high dose of capsaicin \((1.5 \mu \text{g/kg})\) was delivered before, and 5 and 60 min after the perivagal capsaicin treatment.

Spinal cord histology. After the physiological experiments were concluded, C2Hx animals were perfused systemically with heparin-saline followed by 4% paraformaldehyde \((\text{Alfa Aesar})\) and then 10% sucrose in 4% paraformaldehyde. The cervical spinal cord tissue was removed and placed in 30% sucrose in phosphate-buffered saline. The C2 spinal cord segment was cryoprotected and transversely sectioned into \(20-\mu \text{m}\) slices via a Cryostat \((\text{CM 1850, Leica, Germany})\). A subset of animals \((n = 10)\) was perfused with heparin-saline followed by 4% paraformaldehyde and then cut in \(40-\mu \text{m}\) slices with a vibratome \((\text{series 1000, vibratome})\). The spinal cord slices were mounted on glass slides and stained with the crystal violet \((\text{Acros Organics})\). The sections were examined under an upright microscope \((\text{CM850, Leica})\), and photographs were taken with a digital camera \((\text{EOS 600D, Canon})\) connected to the microscope. The cross-sectional ventral and lateral white matter area was manually outlined using ImageJ software. The extent of spared spinal cord tissue in the lesioned side was expressed as a percentage of the ventral and lateral white matter area on the uninjured side at the lesion epicenter. The representative examples of complete and incomplete cervical spinal cord hemisection are presented in Fig. 1.

Data analysis and statistics. The respiratory frequency and tidal volume were calculated from respiratory airflow trace data as previously described (9, 52). In the first experimental protocol, the breathing pattern parameters (e.g., frequency, tidal volume, minute ventilation) were averaged over 10 s before capsaicin injection and analyzed by using a t-test (i.e., uninjured vs. complete C2Hx). Intrajugular capsaicin administration usually induced a prolongation of the expiratory duration (i.e., an apneic response) (Fig. 2); however, a rapid breathing pattern was occasionally observed. This tachypneic response (i.e., the expiratory duration is shorter than 75% baseline after capsaicin injection) was excluded from the
further analysis. Prolongation of the expiratory duration was expressed in an absolute unit (seconds) and as a percentage of the baseline expiratory duration value (%BL). The intensity of the pulmonary chemoreflex was evaluated by the cardiorespiratory response immediately after capsaicin delivery and analyzed by a two-way ANOVA followed by the Student-Newman-Keuls post hoc test [factor 1: group (uninjured vs. complete C2Hx); factor 2: capsaicin dosage (baseline, 0.5, 1, and 1.5 µg/kg)]. To examine whether the spared spinal cord tissue contributed to the recovery of the breathing pattern and pulmonary chemoreflex, linear regression analyses were carried out in which we analyzed the relationship between the extent of spared spinal cord tissue and the respiratory pattern before and after capsaicin administration in complete and incomplete C2Hx animals. In the second experimental protocol, the influence of perivagal capsaicin treatment on the baseline cardiorespiratory pattern and the pulmonary chemoreflex was analyzed by a two-way repeated-measures ANOVA [factor 1: group (uninjured vs. complete C2Hx); factor 2: perivagal capsaicin treatment (before vs. 5 min after treatment)]. All data are expressed as means ± SE. A P value < 0.05 was considered statistically significant.

RESULTS

The pulmonary chemoreflex evoked by activation of bronchopulmonary C-fibers. The representative examples of the breathing pattern before and after capsaicin administration are shown in Fig. 2. Complete C2Hx animals had a significant

---

**Uninjured**

- **Air flow (ml/s)**
  - 1.0 µg/kg
  - 1.5 µg/kg

- **VT (ml)**
  - 1.0 µg/kg
  - 1.5 µg/kg

- **BP (mmHg)**
  - 1.0 µg/kg
  - 1.5 µg/kg

**C2Hx**

- **Air flow (ml/s)**
  - 1.0 µg/kg
  - 1.5 µg/kg

- **VT (ml)**
  - 1.0 µg/kg
  - 1.5 µg/kg

- **BP (mmHg)**
  - 1.0 µg/kg
  - 1.5 µg/kg

---

Fig. 2. Representative examples of capsaicin-induced pulmonary chemoreflexes in an uninjured and a C2 hemisection (C2Hx) animal. The arrow represents the time of capsaicin injection. VT, tidal volume; BP, blood pressure.
higher respiratory frequency and lower tidal volume than uninjured controls 8 wk postsurgery ($P < 0.05$, Table 1). This rapid breathing pattern of C2Hx animals resulted from a reduction in both inspiratory and expiratory duration ($P < 0.05$, Table 1). However, the minute ventilation in complete C2Hx animals was similar to that observed in uninjured animals (Table 1).

Intrajugular capsaicin injection induced a prolongation of the expiratory duration in most animals after moderate (1.0 µg/kg) or high (1.5 µg/kg) dose of capsaicin treatment. Specifically, the expiratory duration was significantly lengthened to $3.3 \pm 0.7$ s (1,005 $\pm$ 201 %BL) and $5.6 \pm 0.9$ s (2,111 $\pm$ 430 %BL) in response to moderate and high doses of capsaicin in uninjured animals, respectively ($P < 0.01$, Fig. 3, A and B). The capsaicin-induced apnea ($2.7 \pm 0.5$ s; 1,182 $\pm$ 300 %BL) was only evident following 1.5 µg/kg capsaicin injection in complete C2Hx animals and was significantly attenuated relative to uninjured animals ($P < 0.01$, Fig. 3, A and B). The high dose of capsaicin induced significant hypotension and bradycardia in uninjured and complete C2Hx animals ($P < 0.05$, Fig. 3, C and D); however, the response of the mean arterial blood pressure and heart rate was similar between two groups of animals ($P > 0.05$, Fig. 3, C and D).

As shown in Fig. 4, breathing patterns were also altered after capsaicin-induced apnea. Specifically, the respiratory cycle was increased following high dose of capsaicin treatment in both uninjured and complete C2Hx animals. The significant prolongation of respiratory cycle lasted for one and three breaths postapnea in uninjured and C2Hx animals, respectively ($P < 0.05$, Fig. 4A). The tidal volume after capsaicin-induced apnea did not change in both uninjured and complete C2Hx groups (Fig. 4B).

The influence of spared spinal cord tissue on the breathing pattern and the pulmonary chemoreflex. Linear regression analyses demonstrated that the amount of white matter tissue sparing in C2Hx animals negatively correlated with respiratory frequency and positively correlated with tidal volume ($P < 0.05$, Fig. 5, A and B) but was not related significantly to minute ventilation (Fig. 5C). Additional linear regression analyses demonstrated that capsaicin (1.0 and 1.5 µg/kg)-induced effects on the expiratory duration (i.e., apnea), blood pressure (i.e., hypotension), and heart rate (i.e., bradycardia) are independent of injury severity when the spare tissue is <50% of contralateral white matter (Fig. 6). When the data points from the animals with minor injury (i.e., spared tissue is ~50–80% of contralateral white tissue) were included for the analysis, a significant but weak correlation between the preserved spinal tissue and intensity of the pulmonary chemoreflex was observed (Fig. 6). These data suggested that there is a minimal spinal tissue sparing threshold needed for spared tissue to support recovery of the pulmonary chemoreflex.

### Table 1. The breathing pattern of uninjured and complete C2Hx animals

<table>
<thead>
<tr>
<th>Breathing Parameter</th>
<th>Uninjured</th>
<th>C2Hx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency, breaths/min</td>
<td>$117 \pm 5$</td>
<td>$150 \pm 11^*$</td>
</tr>
<tr>
<td>Inspiratory duration, s</td>
<td>$0.23 \pm 0.01$</td>
<td>$0.19 \pm 0.01^*$</td>
</tr>
<tr>
<td>Expiratory duration, s</td>
<td>$0.30 \pm 0.02$</td>
<td>$0.23 \pm 0.02^*$</td>
</tr>
<tr>
<td>Tidal volume, ml</td>
<td>$1.8 \pm 0.1$</td>
<td>$1.4 \pm 0.1^*$</td>
</tr>
<tr>
<td>Minute ventilation, ml/min</td>
<td>$203 \pm 10$</td>
<td>$200 \pm 8$</td>
</tr>
</tbody>
</table>

Values are means ± SE. C2Hx, C2 hemisection. *$P < 0.05$, †$P < 0.01$ compared with the uninjured animal.

![Fig. 3](http://jap.physiology.org/figures/1191/Fig3.jpg)
Cardiorespiratory patterns following blockade of background bronchopulmonary C-fiber activity.

Representative examples depicting the influence of bronchopulmonary C-fiber blockade on the cardiorespiratory response in an uninjured and a C2Hx animal are shown in Fig. 7. The respiratory pattern and mean arterial blood pressure during the baseline condition were not affected by perivagal capsaicin treatment in either uninjured controls or complete C2Hx animals (shaded area in Fig. 7 and Fig. 8, A–C). However, the perivagal capsaicin treatment did significantly increase the heart rate in C2Hx animals but not in uninjured animals (P < 0.01, Fig. 8D).

As reported in Fig. 9, capsaicin-induced pulmonary chemoreflex was significantly attenuated when vagal C-fiber conduction was blocked via perivagal capsaicin treatment (P < 0.01, Fig. 9). In uninjured animals, the apneic period was significantly attenuated from 5.3 ± 0.9 s (1,654 ± 332 %BL) to 1.0 ± 0.3 s (299 ± 93 %BL) in uninjured animals. Although the apneic response was weaker in C2Hx animals (2.4 ± 0.3 s; 948 ± 150 %BL) than uninjured animals (P < 0.01, Fig. 9, A and B), it

Fig. 4. Changes in the breathing pattern following the apneic response induced by high dose of capsaicin (1.5 μg/kg). The respiratory cycle remained lengthened after capsaicin-induced apnea in both groups (A); however, the tidal volume was not significantly affected (B). * P < 0.05 compared with BL value. # P < 0.05, significant difference between uninjured and C2Hx animals.

Fig. 5. Relationship between the baseline breathing pattern and the extent of spared ventral and lateral white tissue in C2Hx animals. The results of linear regression analyses are presented for the respiratory frequency (A), tidal volume (B), and minute ventilation (C). The amount of spared tissue is expressed as a percentage of the ventral and lateral white matter area on the contralateral uninjured side (%CL). The light gray circles represent the data points from animals with complete to moderate lesions (i.e., spared tissue <50 %). The dark gray circles represent the data points from animals having more preserved spinal tissue (i.e., spared tissue >50 %). The dashed line represents the trend line when all C2Hx animals were included in the analysis. The solid line represents the trend line when only animals with complete to moderate lesions were included.
Fig. 6. Relationship between the capsaicin-induced pulmonary reflex and the extent of spared spinal tissue in C2Hx animals. Data obtained with the moderate-dose (1.0 µg/kg) injection are shown in A, B, and C. Data obtained with the high-dose (1.5 µg/kg) injection are shown in D, E, and F. Expiratory duration data are in A and D; MAP data are in B and E; heart rate data are in C and F. The light gray circles represent the data points from animals with complete to moderate lesions (i.e., spared tissue <50 %). The dark gray circles represent the data points from animals having more preserved spinal tissue (i.e., spared tissue >50 %). The dashed line represents the trend line when all C2Hx animals were included in the analysis. The solid line represents the trend line when only animals with complete to moderate lesions were included.
remained attenuated by the perivagal capsaicin treatment \((P < 0.01, \text{Fig. 9, A and B})\). Furthermore, the hypotension and bradycardia induced by capsaicin treatment were also abolished by the treatment with perivagal capsaicin in both animal groups \((P < 0.01, \text{Fig. 9, C and D})\).

**DISCUSSION**

There are three major findings of the present study. First, capsaicin-induced pulmonary chemoreflexes could be evoked in chronic C2Hx rats; however, the intensity of the capsaicin-induced apnea was blunted compared with that in uninjured animals. Second, moderate sparing in the spinal cord tissue favored recovery of the baseline breathing pattern but did not influence capsaicin-induced apnea in C2Hx animals. Third, blockade of background vagal C-fiber conduction did not substantially modulate the breathing pattern of C2Hx animals during the resting breathing, suggesting that the rapid shallow breathing pattern following cervical spinal cord injury cannot be attributed to an elevation of background bronchopulmonary C-fiber activity. Together, these findings suggest that C2 spinal hemisection causes a persistent impairment in the respiratory component of the pulmonary chemoreflex and that even moderate white matter sparing is not sufficient to support recovery of the pulmonary chemoreflex.

**Critique of methods.** There is a noteworthy limitation of our methods in the present study, that is, the amount of the spinal tissue spared ipsilateral to the injury was calculated as a percentage of the lateral and ventral white matter area on the uninjured side. We used this criterion because bulbospinal respiratory pathways innervating phrenic motoneurons are located in lateral and ventral white matter \((40)\). The diaphragm is paralyzed after lateral cervical spinal cord injury \((54)\), and recovery of phrenic bursting is positively correlates with the amount of ventromedial tissue spared \((13)\). Thus calculation of spared ventral and lateral white tissue would be expected to be an indicator to predict recovery of the breathing pattern. However, we cannot exclude the possibility that dorsal white matter may also be involved in the respiratory recovery progress given that both phrenic and nonphrenic afferents can influence phrenic activity ipsilateral to a unilateral injury \((55)\).

**Recovery of the pulmonary chemoreflex following chronic spinal cord injury.** Spontaneous recovery of respiratory motor outputs following cervical spinal cord injury has been observed in several studies. Lee et al. \((31)\) observed that the breathing of midcervical hemisected animals can shift from an acute rapid pattern to a more normal rate several weeks postinjury. The tidal volume in spinal cord injured animals can also progressively recover over time postinjury \((9, 31)\). Additionally, our previous study \((32)\) showed that the Hering-Breuer inflation reflex was initially attenuated by cervical spinal hemisection during the subchronic injury phase \((i.e., 2 \text{ wk postinjury})\) but had recovered by 8 wk postinjury. Moreover, intermittent
hypoxia-induced phrenic long-term facilitation can be induced at 8 wk postinjury, but this type of respiratory neuroplasticity was not observed 2 or 4 wk post-C2Hx (7, 14). Although intrajugular capsaicin administration did not trigger the pulmonary chemoreflex at 1 day post-C2Hx (52), the same dose of capsaicin evoked chemoreflexes in C2Hx animals at 8 wk postinjury in the present study. These findings suggest that the pulmonary chemoreflex can recover gradually and partially in C2Hx animals during the chronic injury phase. The accomplishment of the pulmonary chemoreflex depends on the proper

**Fig. 8.** Influence of perivagal capsaicin treatment on the respiratory frequency (A), tidal volume (B), mean arterial blood pressure (C), and heart rate (D) during baseline. *P < 0.05, **P < 0.01, significant difference from the uninjured animals. ##: P < 0.01 significant different from the value before perivagal capsaicin treatment. NS, nonsignificant between the value before and after perivagal capsaicin treatment.

**Fig. 9.** Influence of perivagal capsaicin treatment on the capsaicin-induced pulmonary chemoreflex. Intrajugular capsaicin injection lengthened the expiratory duration (A and B) and reduced the MAP (C) and heart rate (D) in both uninjured controls and C2Hx animals, and these changes in cardiorespiratory parameters were significantly attenuated after perivagal capsaicin treatment. **P < 0.01 compared with the BL value. ##P < 0.01, significant difference between uninjured and C2Hx animals. θθP < 0.01 significant different between the capsaicin-induced response before and after perivagal capsaicin treatment.
signal transmission in the sensory input (i.e., bronchopulmonary C-fibers), central integration area (i.e., nucleus of the solitary tract, respiratory rhythm/pattern generator), and motor output (i.e., spinal respiratory activity). There appears to be a time-dependent change in these elements of the reflex arc after spinal cord injury. For example, Tong et al. (51) demonstrated that c-fos expression in neurons within nucleus of the solitary tract after gastrointestinal vagal afferent activation was attenuated 3 days postinjury but recovered by 3 wk postinjury in thoracic spinal cord-injured rats. In addition, a gradual recovery of 5-HT innervations and upregulation of 5-HT2A receptors in the phrenic nucleus may contribute to recovery of phrenic motor output and neuroplasticity in chronic C2Hx animals (11, 14). Moreover, changes in glutamate receptor subunit expression in the medulla and spinal cord may be involved in regulation of the respiratory recovery (1, 57). Hence, a combination of peripheral and central factors could support the recovery of pulmonary chemoreflex during the chronic injury phase.

Although the pulmonary chemoreflex could be induced in chronic C2Hx animals, we noticed that the capsaicin-induced apneic response remained impaired. However, the degree of hypotension and bradycardia observed in C2Hx animals is similar to uninjured animals. Kuo and Lai (24) observed that ovalbumin-induced sensitization of bronchopulmonary C-fiber only augmented the apneic response following intrajugular capsaicin injection. Similarly, although chronic cigarette smoke exposure is associated with prolonged capsaicin-evoked apnea, it does not produce a significant effect on the cardiovascular reflex response (44). These findings suggest that the respiratory and cardiovascular components of the pulmonary chemoreflex are differentially modulated and may have distinct recovery time courses after spinal cord injury. In addition, our previous study (32) demonstrated that the Hering-Breuer inflation reflex fully recovered by 8 wk post-C2Hx; however, the pulmonary chemoreflex remained weaker than uninjured control animals. Although both reflexes induce an apneic response, the induction of the reflex is mediated by distinct vagal afferents. The inflation reflex is primarily mediated by myelinated fibers (i.e., slowly adapting receptors), whereas the pulmonary chemoreflex is mainly evoked by activation of unmyelinated afferents (i.e., bronchopulmonary C-fibers). Therefore, recovery of the apneic response may also depend on afferent input signal type and the corresponding central mechanism.

**Contribution of the spared spinal cord tissue to the breathing pattern and the pulmonary chemoreflex in chronically C2Hx animals.** In the present study, tidal volume positively correlated with the extent of spared spinal tissue in chronic cervical spinal cord-injured animals. Several potential mechanisms may contribute to the recovery of tidal volume in animals with incomplete lesions. First, it has been reported that the phrenic nucleus is innervated not only by the ipsilateral bulbospinal pathway from the ventral respiratory group but also the contralateral descending pathway located in the ventromedial and ventral funiculus (40). Thus activation of these crossed phrenic pathways could enhance recovery of respiratory motor outputs ipsilateral to the lesion (13). Second, some ipsilateral respiratory pathways are located in the medial part of the spinal cord and would therefore not be directly interrupted by a lateral spinal cord injury. Vinit et al. (53) proposed that these preserved ipsilateral medial respiratory pathways could be activated during chronic injury phase and thereby support respiratory recovery in C2Hx animals. Third, Lane et al. (26) demonstrated that there are a small number of prephrenic interneurons located rostral to the phrenic nucleus. Some of these spinal interneurons receive projections from the ventral respiratory group and exhibit respiratory-modulated bursting (25). Accordingly, interneurons within spared grey matter may be also involved in the regulation of the tidal volume in chronic spinal injury. Finally, spared spinal tissue may also modulate the uninjured respiratory output (i.e., spinal respiratory motor outputs contralateral to the hemisection) and provide a substrate for compensatory plasticity after lateral spinal cord injury (20).

Linear regression analyses suggested that there is a significant correlation between the intensity of the pulmonary chemoreflex and the extent of spared spinal tissue in C2Hx animals when including all data points from complete and incomplete C2Hx animals. However, the squared correlation coefficient ($r^2$) is weak (i.e., <0.2; Fig. 6), suggesting influence of the spared tissue on the recovery of the pulmonary chemoreflex following spinal cord injury remained to be determined. The potential mechanism underlying this correlation remains unclear; we suspect that recovery of the normal breathing pattern may be of benefit to the expression of the pulmonary defense reflex. Alternatively, the pulmonary chemoreflex may not be influenced in animals with minor cervical injury.

**Role of the bronchopulmonary C-fiber on the cardiorespiratory pattern following chronic spinal cord hemisection.** The bronchopulmonary C-fibers are the primary chemosensitive afferents in the lung and are involved in regulating the breathing pattern during physiological and pathological conditions (35, 36). Their baseline discharge is usually low. However, blockade of vagal C-fiber conduction with a perivagal capsaicin treatment has been reported to enhance tidal volume and reduce respiratory frequency in anesthetized dogs, suggesting that background vagal C-fiber activity can influence the respiratory pattern at the rest (33, 50). Although several studies have indicated that the baseline cardiorespiratory pattern appears to be unaffected by the perivagal capsaicin treatments under normal breathing conditions (17, 34), background vagal C-fiber activity has been shown to contribute to the rapid shallow breathing under experimental pathological conditions (e.g., pulmonary vascular congestion and pulmonary air embolism) (6, 18). Golder et al. (15) demonstrated that cervical spinal hemisection induced a rapid shallow breathing pattern in anesthetized rat at 1 and 2 mo postinjury, and this breathing pattern of injured animals was not different from uninjured animals after bilateral cervical vagotomy. This result suggests that vagal afferents may contribute to the alteration of the breathing pattern following cervical spinal cord injury. Here, we showed that the breathing pattern of C2Hx animals was not changed by blockade of vagal C-fibers, suggesting that background activity of another type of vagal afferent (e.g., slowly adapting receptors and/or rapidly adapting receptors) may have a predominant role in regulating respiratory function after spinal cord injury in the rat.

Although perivagal capsaicin treatment did not affect breathing pattern in either group, it did significantly increase the heart rate in C2Hx animals, indicating that vagal C-fiber afferents may tonically depress the heart rate in the chronic injury phase. Jones et al. (21) had demonstrated that some
cardiac vagal preganglionic neurons within the dorsal vagal motor nucleus have unmyelinated axons (i.e., C-fibers), and selective stimulation of cardiac vagal C-fiber efferents can also induce bradycardia (21, 22). Therefore, cervical spinal cord injury may result in an augmentation of background vagal C-fiber efferent activity that inhibits cardiac activity.

**Functional significance.** Attenuation of the efficacy of the pulmonary defense reflex has been associated with greater pulmonary morbidity in patients with chronic spinal cord injury. The pulmonary chemoreflex can prevent inhalation of respiratory irritants and reduce further damage to the pulmonary system. Our present result demonstrated that although the pulmonary chemoreflex can recover following spinal cord injury, it remains relatively weak compared with uninjured animals. Although the presence of preserved spinal tissue contributed to tidal volume generation, it did not enable expression of the pulmonary chemoreflex in incomplete C2Hx animals. That is, the pulmonary chemoreflex remained impaired even in animals with moderate spinal tissue sparing. This result corresponds with the clinical observation of patients with spinal cord injury having a reduced ability to produce airway protective behaviors even after they have recovered spontaneous breathing (3). Accordingly, whether therapeutic approaches focusing the spinal cord repair can improve the recovery of the pulmonary chemoreflex remains to be examined, and the development of treatment targeted on the nonspinal level (i.e., regulation of afferent sensitivity and/or central respiratory drives) may have a potential to enhance recovery of the pulmonary defense reflex.

**GRANTS**

Support for this work was provided by grants from the National Health Research Institutes (NHRI-EX103-10223NC), National Science Council (NSC 100–2320-B-110–003-MY2 and NSC 102–2320-B-110–004-MY3), and NSYSU-KMU Joint Research Project (2014-I006).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: K.-Z.L. and Y.-S.C. performed experiments; K.-Z.L. and Y.-S.C. analyzed data; and K.-Z.L. and Y.-S.C. interpreted results of experiments; K.-Z.L. and Y.-S.C. and Y.-S.C. contributed to tidal volume generation, it did not enable expression of the pulmonary chemoreflex in incomplete C2Hx animals. That is, the pulmonary chemoreflex remained impaired even in animals with moderate spinal tissue sparing. This result corresponds with the clinical observation of patients with spinal cord injury having a reduced ability to produce airway protective behaviors even after they have recovered spontaneous breathing (3). Accordingly, whether therapeutic approaches focusing the spinal cord repair can improve the recovery of the pulmonary chemoreflex remains to be examined, and the development of treatment targeted on the nonspinal level (i.e., regulation of afferent sensitivity and/or central respiratory drives) may have a potential to enhance recovery of the pulmonary defense reflex.

**REFERENCES**