Respiratory motor outputs following unilateral midcervical spinal cord injury in the adult rat

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Submitted 3 September 2013; accepted in final form 22 November 2013

CERVICAL SPINAL CORD INJURY is always associated with impaired respiratory function (1, 36, 44), which is the major cause of mortality and morbidity in spinal cord-injured patients (3, 6). The diaphragm is one of the primary respiratory muscles in mammals and is innervated by the phrenic motoneuron pool located at the C3-C6 spinal cord (19, 24). Cervical spinal cord injury usually causes respiratory insufficiency and complications due to the interruption of the bulbospinal respiratory pathway and/or direct damage of phrenic motoneurons (15, 20, 21, 39). The most extensively documented cervical spinal cord injury animal model is the unilateral hemisection at the highcervical segment [e.g., C3 hemisection (C3Hx)] (15, 20, 21). This injury model causes immediate inactivation of the phrenic nerve, paralysis of the hemidiaphragm, and reduction of tidal volume in the adult rat (9, 33, 38). Over weeks to months postinjury, both phrenic motor outputs (e.g., inspiratory phrenic and diaphragmatic activity) and respiratory behaviors (e.g., tidal volume) can be partially recovered through the activation of a latent crossed respiratory pathway (i.e., crossed phrenic phenomenon) (9, 11, 15, 21, 23, 26, 33, 40). Various pharmacological and physiological approaches have been used to enhance this intraspinal neuroplasticity to improve respiratory functions following C3Hx in that animal model (12, 14, 17, 28, 41, 42).

The majority of human spinal cord injury occurs at the midcervical level (C2-C3); therefore, the changes in respiratory function after midcervical spinal cord injury have attracted increasing scientific and clinical attention (2, 13, 19, 20, 30, 34, 35). Midcervical spinal cord injury not only interrupts the bulbospinal respiratory pathway, but also directly damages phrenic motoneurons. Golder et al. (13) demonstrated that midcervical (C3/4) contusion induced rapid, shallow breathing in adult rats at 2 days postinjury, and the breathing pattern returned to the control value at 2 wk postinjury. Similar respiratory dysfunction was also observed after C5 hemicontusion (5). Our laboratory’s recent report showed that the breathing pattern was not substantially altered by C3/4 spinal cord contusion injuries (22). This discrepancy in the respiratory pattern suggests that the impact of midcervical contusion injury on respiration is variable and may depend on the difference in the injury severity in the white vs. gray matter (22). Although contusion injury models are more clinically relevant, the hemisection lesion model has the advantages of surgical precision, anatomical specificity, and consistent reproducibility. In addition, the difference in the neuroplasticity of the motor outputs on the contralateral (i.e., uninjured) and ipsilateral (i.e., injured) side can be compared in the unilateral spinal cord hemisection model. Midcervical hemisection has been used to investigate alterations to locomotor function following spinal injury (18, 30, 31, 37); however, changes in breathing patterns following midcervical hemisection have not yet been defined. Accordingly, the primary purpose of the present study was to investigate the impact of midcervical hemisection [i.e., C3 hemisection (C3Hx)] on phrenic motor outputs and compare respiratory functional recovery following high- vs. midcervical spinal cord hemisection. We hypothesized that C3Hx would induce a similar rapid, shallow breathing pattern to C2Hx in the acute injury phase (1 day to 1 wk) due to the loss of a portion of the phrenic motoneurons; however, sparing the phrenic motoneurons and bulbospinal respiratory pathways above the hemisection would enable a greater and faster respiratory recovery in C3Hx animals. In addition, the phrenic burst amplitude ipsilateral to the lesion would be reduced in both C2Hx and C3Hx animals; however, the capacity to enhance activity during higher respiratory drives would be blunted following midcervical spinal cord injury.
MATERIALS AND METHODS

Animals

A total of 47 adult male Sprague-Dawley rats obtained from BioLasco Taiwan were divided into the following groups: naive (n = 4); C2 laminectomy (n = 8); C3/4 laminectomy (n = 3); C2Hx (n = 17), and C4Hx (n = 15). There were no significant differences in the body weight and cardiorespiratory patterns between the naive animals and those rats receiving laminectomy surgery (two-way repeated-measures analysis of variance (RM-ANOVA), factor 1: animal group (naive, C2 laminectomy, C3/4 laminectomy); factor 2: time point (1 day and 1, 2, 4, and 8 wk postinjury)); therefore, these animals were combined as a single control group (n = 15). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the National Sun Yat-sen University.

Spinal Cord Injury

Spinal cord injury and laminectomy surgery were performed at 9 wk of age (64.0 ± 0.2 days, mean ± SE). The animals were anesthetized with xylazine (10 mg/kg ip, Rompun, Bayer) and ketamine (140 mg/kg ip, Ketalar, Pfizer) and then placed in the prone position. A dorsal cervical incision was made from the C1 to C3 spinal cord, followed by C2 laminectomy in the animals of the C2Hx and C2 laminectomy groups. A portion of the C3 and C4 vertebral bone was removed to expose the C4 spinal cord in the animals of the C4Hx and C3/4 laminectomy groups. A left C2Hx or C4Hx was then performed using a microscalpel and gentle aspiration in the animals of the C2Hx and C3/4Hx groups, respectively. The dura and overlying muscles were sutured with 10–0 nylon (UNIK) and 4–0 chronic (UNIK) sutures, respectively. The skin was subsequently closed with 4–0 nylon sutures (UNIK). Following the surgery, the animals were given injections of yohimbine (1.2 mg/kg sc, Tocris) to reverse the effect of xylazine, lactated Ringer solution (5 ml sc, Nang Knue Pharmaceutical) to prevent dehydration, and buprenorphine (0.03 mg/kg sc, Shinlin Sinseng Pharmaceutical) for analgesia. The postsurgical care protocol, including daily oral supply of Nutri-cal (1–3 ml, EVSCO Pharmaceuticals) and injection of lactated Ringer solution (5 ml sc), was applied until adequate volitional drinking and eating were recovered.

Whole Body Plethysmography in Unanesthetized Rats

Respiratory behaviors (e.g., tidal volume, respiratory frequency, and minute ventilation) of unrestrained, unanesthetized animals were measured using the whole body plethysmography system (Buxco Research Systems) at 1 day and 1, 2, 4, and 8 wk postsurgery. The chamber flow, temperature, and humidity were calibrated by the standard procedure, and the body temperature of the animals was measured on the day of the experiment. The animal was placed in a Plexiglas chamber (no. PLY4213, volume: 3.9 liters) and exposed to normoxic gas (21% O2, balance N2) for 60 min to establish the baseline measurements and then hypercapnic gas (7% CO2, 21% O2, balance N2) for 10 min by flushing compressed gas mixtures (2.5 l/min) into the chamber. After the hypercapnic challenge, the animals were exposed to normoxic gas for 10 min and then removed from the chamber and placed back in the cage.

Phrenic Nerve Recording in Anesthetized and Ventilated Rats

A subset of animals (control, n = 11; C2Hx, n = 13; C4Hx, n = 13) were used to evaluate phrenic motor outputs at 8–9 wk postinjury. The animals were anesthetized with urethane (1.6 g/kg ip, Sigma) and placed in a supine position. The rectal temperature was monitored by an electrical thermometer and maintained at 37 ± 1° by a servo-controlled heating pad (model TC-1000, CWE). The trachea was cannulated below the larynx with the endotracheal tube (PE-240, Clay Adams). The femoral artery was catheterized (PE-50) for blood pressure measurement (transducer: DTX-1; amplifier: TA-100, CWE). Another PE-50 catheter was inserted into the femoral vein for drug administration. The animals were then mechanically ventilated (KDS 35, KD Scientific) with an oxygen/nitrogen mixture (50% O2, balance N2; volume = 7 ml/kg; frequency = 60–70/min) and paralyzed with pancuronium bromide (2.5 mg/kg iv, Fresenius Kabi). The blood gas was not analyzed in the present study, but the partial pressure of end-tidal CO2 (PetCO2) was monitored with a Capnogard CO2 monitor (Novametrix Medical Systems) by placing a CO2 sensor on the expiratory line of the ventilator circuit. Our laboratory’s previous study showed that vagal inputs have robust inhibitory effects on the phrenic bursting ipsilateral to the lesion (26); therefore, the PetCO2 was maintained at 50 Torr to confirm that the weak ipsilateral phrenic bursting under the vagal intact condition was not due to low PetCO2 by adjusting the ventilator rate and/or the inspired CO2 throughout the experiment.

The bilateral phrenic nerves were isolated and sectioned distally in the cervical region via a ventral approach (9, 26). The phrenic nerve activity was recorded by a monopolar silver electrode (no. 782500, A-M Systems) and then amplified (×1,000) and band-pass filtered (0.3–10 kHz) by a differential A/C amplifier (model 1700, A-M Systems). The neural signals were digitized by the CED Power 1401 data acquisition interface and processed with the rectify and smooth function (time constant: 25 ms) of Spike2 software (Cambridge Electronic Design, Cambridge, UK). All signals were stored on a PC for the offline analysis. After stable recording of the bilateral phrenic nerves for 10 min, bilateral cervical vagotomy was performed to investigate changes in bilateral phrenic nerve activity after removal of vagal inputs. The PetCO2 was then adjusted to 85 Torr for 5 min by raising the inspired CO2 concentration when phrenic burst amplitude reached a plateau under the vagotomized condition. Ten minutes after the hypercapnic challenge, asphyxia was applied by turning off the ventilator for ~1 min.

Spinal Cord Histology

At the end of the neurophysiology protocols, the animals were systemically perfused with heparin-saline (5.5 IU/ml), followed by

Fig. 1. Representative histological examples of a C2 (A) and C3 (B) spinal hemisection (Hx).
Values are means ± SE in g. C2Hx and C4Hx animals, respectively. *P < 0.05 and **P < 0.01, significant differences from control animals. #P < 0.05, significant differences between C2Hx and C4Hx animals. aP < 0.01 compared with the value at 1 day postinjury. bP < 0.01 compared with the value at 1 day and 1 wk postinjury. cP < 0.01 compared with the value at 1 day and 1–2 wk postinjury. dP < 0.01 compared with the value at 1 day and 4 wk postinjury.

4% paraformaldehyde (Alfa Aesar). The cervical spinal cord was removed, cryoprotected, and cut into 40-μm sections using a vibratome (Vibratome 1000). Some animals were perfused with hepa-
rin-saline, followed by 4% paraformaldehyde and then 10% sucrose in 4% paraformaldehyde. The cervical spinal cord tissue was removed, cryoprotected, and cut into 40-
m sections using a vi-
bratome (Vibratome 1000). Some animals were perfused with hepa-
rin-saline, followed by 4% paraformaldehyde and then 10% sucrose in 4% paraformaldehyde.

Data Analyses

Whole body plethysmography in unanesthetized rats. The whole body plethysmography data (e.g., tidal volume, respiratory frequency, and minute ventilation) were analyzed using FinePointe software (Buxco Research Systems). The respiratory frequency data were derived from the airflow trace, and respiratory tidal volume and minute ventilation were calculated by the Drorbaugh and Penn equa-
tions (10). All data were exported to an Excel file; the data for the stable 10 min were averaged for the baseline recording, and the data for the last 2 min were averaged for the hypercapnic challenge recording. Respiratory tidal volume and minute ventilation were expressed as absolute values (ml, ml/min) or were normalized to body weight (ml/100 g and ml/100 g · min⁻¹). The respiratory parameters and body weight data were analyzed by the two-way RM-ANOVA [factor 1: animal group (control, C2Hx, C4Hx); factor 2: time point (1 day and 1, 2, 4, and 8 wk postinjury)] followed by the Student-
Newman-Keuls post hoc test. Linear regression analyses were used to compare recovery progress of tidal volume between C2Hx and C4Hx animals during the baseline condition and hypercapnia.

Phrenic nerve recording in anesthetized and ventilated rats. In-
spiratory duration (Ti), expiratory duration (Te), and respiratory cycle were calculated by the rectified and smoothed phrenic neurogram. The Ti was determined as the period between inspiratory phrenic onset and the time point when integrated phrenic amplitude declined to 50% of the peak value, as previously described (25). The Ti was defined as the interval between the end of the Ti and the onset of the subsequent inspiratory burst. The respiratory frequency was calculated as 60/ (Ti + Te). The phrenic burst amplitude was defined as the difference between the maximum and minimum value of the processed phrenic neurogram within a single neural breath. The data were averaged over 30 s under baseline, vagotomized, and hypercapnic conditions. The maximum phrenic burst amplitude was also assessed during the asphyxic response. The phrenic burst amplitude was expressed in arbitrary units (a.u.) and analyzed by using one-way ANOVA followed by the Student-Newman-Keuls post hoc test.

Fig. 2. Representative examples of the airflow trace recorded from a control, C2Hx, and C4Hx rat during the baseline condition and hypercapnia at 1 wk and 8 wk postinjury.
All data are presented as means ± SE. A P value of < 0.05 is considered statistically significant for all analyses.

RESULTS

Body Weight

The body weight of all animals was similar between the different groups at 1 day postsham or hemisection surgery (Table 1). The body weight was significantly reduced after the hemisection surgery in both the C2Hx and C4Hx group from 1 to 8 wk postinjury (P < 0.05, Table 1); however, the weight gradually recovered in both injury groups after 1 wk postinjury. At 8 wk postinjury, the weights of the animals of the C4Hx group were slightly greater than that of the C2Hx animals (P = 0.046, Table 1).

Respiratory Behaviors in Unanesthetized Rats

Representative examples of the airflow trace recorded by whole body plethysmography at 1 and 8 wk postinjury are shown in Fig. 2. During the baseline breathing measurement (i.e., 21% O2, balance N2), the respiratory frequency was significantly higher in the C2Hx (136 ± 5 breaths/min) and C4Hx (134 ± 6 breaths/min) animals than the control animals.

Table 2. TI and TE of unanesthetized control, C2Hx, and C4Hx animals during the baseline

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>1 wk</th>
<th>2 wk</th>
<th>4 wk</th>
<th>8 wk</th>
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</thead>
<tbody>
<tr>
<td>TI</td>
<td>0.27 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>C2Hx</td>
<td>0.25 ± 0.01</td>
<td>0.22 ± 0.01**</td>
<td>0.20 ± 0.01***</td>
<td>0.20 ± 0.01***</td>
<td>0.20 ± 0.02***</td>
</tr>
<tr>
<td>C4Hx</td>
<td>0.25 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.24 ± 0.01##</td>
<td>0.24 ± 0.01#</td>
</tr>
<tr>
<td>TE</td>
<td>0.32 ± 0.02</td>
<td>0.36 ± 0.02*</td>
<td>0.38 ± 0.02*</td>
<td>0.38 ± 0.02*</td>
<td>0.37 ± 0.02*</td>
</tr>
<tr>
<td>C2Hx</td>
<td>0.20 ± 0.01**</td>
<td>0.25 ± 0.01***</td>
<td>0.24 ± 0.01***</td>
<td>0.26 ± 0.01***</td>
<td>0.25 ± 0.01***</td>
</tr>
<tr>
<td>C4Hx</td>
<td>0.21 ± 0.01**</td>
<td>0.28 ± 0.01***</td>
<td>0.30 ± 0.01***#</td>
<td>0.33 ± 0.02###</td>
<td>0.35 ± 0.02###</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE in s. TI, inspiratory duration; TE, expiratory duration. *P < 0.05 and **P < 0.01 compared with control animals. #P < 0.05 and ##P < 0.01, significant differences between C2Hx and C4Hx animals. +P < 0.05 compared with the value at 1 day postinjury. **P < 0.05 compared with the value at 1 day and 1–2 wk postinjury.
(105 ± 4 breaths/min) at 1 day postinjury (P < 0.01, Fig. 3). Postinjury, the respiratory frequency of the C4Hx animals gradually returned to normal over time; however, the C2Hx animals still maintained a higher respiratory frequency than both the control and C4Hx animals at 8 wk postinjury (P < 0.01, Fig. 3). A higher respiratory frequency was observed for the animals in both injured groups at 1 day postinjury, primarily due to the shortening of the Td (P < 0.01, Table 2). The Td also decreased in the C2Hx animals at 1–8 wk postinjury (P < 0.01, Table 2). Both the C2Hx and C4Hx animals demonstrated a considerably lower tidal volume compared with the control animals at all postinjury time points (P < 0.01, Fig. 3). Although the tidal volume was similar in the C2Hx and C4Hx animals at the acute injury time point, a greater progressive recovery was observed in the C4Hx animals. In particular, the C4Hx animals had higher tidal volumes than the C2Hx animals during the baseline condition; however, the capability to enhance tidal volume during respiratory challenge (i.e., hypercapnia) remained impaired in these animals.

During hypercapnia, the respiratory frequency, tidal volume, and minute ventilation increased for the animals in all groups, as expected (Figs. 2 and 3). There was no significant difference in the respiratory frequency across the groups; however, both the tidal volume and minute ventilation remained lower in the C2Hx and C4Hx animals than in the control animals at all postinjury time points (Fig. 3). In addition, the C4Hx animals did not show a greater tidal volume than the C2Hx animals until 8 wk postinjury (Fig. 3).

To evaluate the relationship between tidal volume and time postinjury, linear regression analyses were used to compare the recovery progress between the C2Hx and C4Hx animals (Fig. 4). The slope of the regression line for the data of the C4Hx animals was significantly greater than that of the C2Hx animals during the baseline condition, but not hypercapnia (Fig. 4). These results suggested that the C4Hx animals exhibited a better tidal volume recovery progress than the C2Hx animals during the baseline condition; however, the capability to enhance tidal volume during respiratory challenge (i.e., hypercapnia) remained impaired in these animals.

Table 3. The tidal volume and minute ventilation normalized by the body weight at different time points postinjury

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>1 wk</th>
<th>2 wk</th>
<th>4 wk</th>
<th>8 wk</th>
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<tbody>
<tr>
<td><strong>Tidal volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.47 ± 0.02</td>
<td>0.52 ± 0.02</td>
<td>0.50 ± 0.02</td>
<td>0.51 ± 0.02</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>C2Hx</td>
<td>0.39 ± 0.01*</td>
<td>0.40 ± 0.01**</td>
<td>0.37 ± 0.01**</td>
<td>0.36 ± 0.01**</td>
<td>0.33 ± 0.01**</td>
</tr>
<tr>
<td>C4Hx</td>
<td>0.42 ± 0.01*</td>
<td>0.45 ± 0.02**#</td>
<td>0.43 ± 0.02**##</td>
<td>0.42 ± 0.01**##</td>
<td>0.40 ± 0.02###</td>
</tr>
<tr>
<td><strong>Hypercapnia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.80 ± 0.03</td>
<td>0.85 ± 0.04</td>
<td>0.79 ± 0.04</td>
<td>0.81 ± 0.03</td>
<td>0.77 ± 0.04</td>
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<tr>
<td>C2Hx</td>
<td>0.54 ± 0.02**</td>
<td>0.70 ± 0.05**</td>
<td>0.57 ± 0.03**</td>
<td>0.57 ± 0.03**</td>
<td>0.49 ± 0.03**</td>
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<tr>
<td>C4Hx</td>
<td>0.59 ± 0.03**</td>
<td>0.73 ± 0.03**</td>
<td>0.63 ± 0.04**</td>
<td>0.62 ± 0.02**</td>
<td>0.59 ± 0.03**#</td>
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<tr>
<td><strong>Minute ventilation</strong></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>48.7 ± 1.6</td>
<td>50.6 ± 1.6</td>
<td>48.8 ± 2.3</td>
<td>47.4 ± 2.2</td>
<td>46.1 ± 3.5</td>
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<tr>
<td>C2Hx</td>
<td>53.1 ± 1.8</td>
<td>51.4 ± 1.1</td>
<td>50.7 ± 1.6</td>
<td>49.3 ± 1.4</td>
<td>44.5 ± 1.4</td>
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<tr>
<td>C4Hx</td>
<td>55.3 ± 3.2</td>
<td>51.7 ± 1.3</td>
<td>49.3 ± 1.4</td>
<td>44.5 ± 1.4</td>
<td>43.2 ± 2.7</td>
</tr>
<tr>
<td><strong>Hypercapnia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>139 ± 7</td>
<td>158 ± 10</td>
<td>156 ± 9</td>
<td>152 ± 9</td>
<td>146 ± 10</td>
</tr>
<tr>
<td>C2Hx</td>
<td>85 ± 3**</td>
<td>134 ± 7**</td>
<td>118 ± 5**</td>
<td>117 ± 6**</td>
<td>104 ± 4**</td>
</tr>
<tr>
<td>C4Hx</td>
<td>92 ± 6**</td>
<td>133 ± 6**</td>
<td>125 ± 6**</td>
<td>123 ± 7**</td>
<td>116 ± 7**</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml/100 g for tidal volume and in ml/min/100 g for minute ventilation. *P < 0.05 and **P < 0.01 compared with control animals. #P < 0.05 and ##P < 0.01, significant differences between C2Hx and C4Hx animals.
The tidal volume and minute ventilation were normalized to the body weight and are presented in Table 3. The results were similar to those presented for the raw data values in Fig. 3.

Phrenic Motor Outputs in Anesthetized and Ventilated Rats

Representative examples of bilateral phrenic neurograms recorded in a control and C2Hx and C4Hx animal under the baseline, vagotomized, and hypercapnic conditions. Bilateral phrenic nerve activity is presented as the raw signals (Phr) and the rectified and moving averaged signals (JPhr). Bilateral phrenic neurogram amplitude is similar in the control animal, but IL [ipsilateral (i.e., injured or left side)] phrenic burst amplitude is reduced compared with the CL [contralateral (i.e., uninjured or right side)] phrenic nerve in both C2Hx and C4Hx animals.

The tidal volume and minute ventilation were normalized to the body weight and are presented in Table 3. The results were similar to those presented for the raw data values in Fig. 3.

**Table 3.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vagotomy</th>
<th>Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL Phr</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>IL Phr</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>CL Phr</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>IL Phr</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**Fig. 5.** Representative bilateral phrenic neurograms recorded in a control, C2Hx, and C4Hx animal under the baseline, vagotomized, and hypercapnic conditions. Bilateral phrenic nerve activity is presented as the raw signals (Phr) and the rectified and moving averaged signals (JPhr). Bilateral phrenic neurogram amplitude is similar in the control animal, but IL [ipsilateral (i.e., injured or left side)] phrenic burst amplitude is reduced compared with the CL [contralateral (i.e., uninjured or right side)] phrenic nerve in both C2Hx and C4Hx animals.
condition \((P < 0.05, \text{Fig. 7})\) and greater ipsilateral phrenic burst amplitudes than the C2Hx animals under all conditions \((P < 0.01, \text{Fig. 7C})\).

Although bulbospinal respiratory pathways innervating the contralateral phrenic motoneuron pool were not directly affected by the surgery, neuroplasticity associated with the unilateral spinal hemisection may also modulate contralateral phrenic bursting. Our present data demonstrated that the contralateral phrenic burst amplitude was slightly greater in the C2Hx \((0.25 \pm 0.02 \text{ a.u.})\) and C4Hx \((0.23 \pm 0.02 \text{ a.u.})\) animals than in the control \((0.16 \pm 0.02 \text{ a.u.})\) animals during the baseline condition \(\text{(one-way ANOVA, } P < 0.05)\), suggesting a compensatory response may be evoked after cervical spinal cord injury. In addition, the relative increase in the contralateral phrenic burst amplitude \((%BL)\) was attenuated in both injured groups compared with the control group under the vagotomized and asphyxic conditions \((P < 0.05, \text{Fig. 7C})\).

**Mean Arterial Blood Pressure and Heart Rate**

The mean arterial blood pressure was similar between the groups during the baseline condition \((P > 0.05, \text{Table 5})\) and was significantly increased in all groups during hypercapnia \((P < 0.01, \text{Table 5})\). The heart rate was similar between the groups during all conditions \((P > 0.05, \text{Table 5})\).

**DISCUSSION**

The present study demonstrated that midcervical spinal cord hemisection caused a significant respiratory insufficiency indicated by a rapid, shallow breathing pattern during the acute injury phase (i.e., 1 day postinjury) and a reduction of tidal volume from 1 to 8 wk postinjury. In addition, the bilateral phrenic motor outputs were altered following C4Hx. Specifically, the phrenic burst amplitude ipsilateral to the lesion was attenuated in the C4Hx animals compared with the control animals. The relative increase in the contralateral phrenic burst amplitude during the higher respiratory drives (e.g., vagotomy

![Fig. 6. Representative bilateral phrenic neurograms recorded in a control, C2Hx, and C4Hx animal during asphyxia. Bilateral phrenic motor outputs were enhanced during cessation of mechanical ventilation (labeled by the horizontal dotted line) in all group animals.](image)

**Table 4.** Respiratory frequency of anesthetized and ventilated animals during the baseline, vagotomized, and hypercapnic condition

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Vagotomy</th>
<th>Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63 ± 2</td>
<td>40 ± 2*</td>
<td>42 ± 2*</td>
</tr>
<tr>
<td>C2Hx</td>
<td>62 ± 1</td>
<td>46 ± 2**</td>
<td>45 ± 2*</td>
</tr>
<tr>
<td>C4Hx</td>
<td>65 ± 1</td>
<td>45 ± 2*</td>
<td>45 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE in bursts/min. *\(P < 0.05\) compared with the control animals. **\(P < 0.05\) compared with the baseline value.
and asphyxia) was blunted following chronic C4Hx. We also observed that the respiratory functional recovery and phrenic motor outputs were distinct between the C2Hx and C4Hx animals in several aspects. First, the respiratory frequency in the unanesthetized C2Hx animals was significantly increased throughout the entire recording period (i.e., 1 day to 8 wk postinjury); however, it gradually returned to normal in the C4Hx animals. Second, the tidal volume recovery progress was greater in the C4Hx animals than in the C2Hx animals during the baseline condition (21% O2, balance N2) but not during hypercapnia (7% CO2, 21% O2, balance N2). Third, the relative increase in the ipsilateral phrenic burst amplitude (%BL) during the higher respiratory drives was significantly lower in the C4Hx animals than in the C2Hx animals; even the raw burst amplitude (a.u.) was higher in the C4Hx animals. Taken together, these results demonstrate that high- and midcervical spinal cord injury has a differential impact on breathing function and phrenic motor output.

**Critique of Methods**

One potential concern of our experimental approach should be addressed in the present study. Because vagal afferent inputs have an inhibitory impact on the ipsilateral phrenic activity, the baseline PETCO2 was maintained at 50 Torr to ensure the ipsilateral phrenic bursting under vagal-intact status.
However, relative higher PETCO₂ may activate phrenic motoneurons, which were originally inactive under the normal condition (23). Therefore, the ipsilateral phrenic bursting amplitude may be overestimated in the present study.

**Impact of Midcervical Spinal Cord Injury on Breathing Pattern and Phrenic Motor Outputs**

Several studies have used the contusion model to investigate the impact of midcervical spinal cord injury on respiratory function (5, 13, 22, 34). Choi et al. (5) demonstrated that C₅ lateral contusion caused a lesion severity-dependent respiratory deficit indicated by a rapid, shallow breathing pattern and a significant diminished ability to enhance ventilation during hypercapnic challenge (7% CO₂, 90% O₂, balance N₂). However, the breathing pattern returned to normal at 6 wk postinjury (5). A similar transient rapid, shallow breathing pattern was also observed following C₄₅/S contusion (13) or C₄ lateral contusion (34). Our recent report indicated that ventilatory behaviors of unanesthetized animals were not significantly influenced by midline midcervical (C₃₄S) contusion (22). These studies suggest that midcervical contusion injury causes transient respiratory deficits, but that compensatory respiratory responses may be evoked to maintain the normal ventilation from days to weeks postinjury (16). Unlike the full recovery of tidal volume observed in the midcervical contusion animal model, the present data showed that midcervical hemisection induced a long-term reduction in tidal volume, which is similar to previous observations of tetraplegic patients (4). The rat phrenic nucleus is located in the ventral horn of the C₃ to C₆ spinal cord and receives bulbospinal respiratory projections via the lateral and ventral funiculi (19, 27). The hemisection of the lateral and ventral funiculi (19, 27). The hemisection may have also contributed to the blunted phrenic motor response to respiratory challenge.

**Recovery of Respiratory Behaviors Following Midcervical Spinal Cord Injury**

The present study demonstrated that C₄Hₓ caused a transient rapid, shallow breathing pattern from 1 day to 1 wk postinjury. During 2–8 wk postinjury, the tidal volume remained lower in the C₄Hₓ animals than the control animals, but the respiratory frequency returned to the control value. The analysis of respiratory cycle duration indicated that the recovery of respiratory frequency was primarily due to a progressive elongation of Tₑ. Several studies have shown that spinal cord injury induces a concomitant change of the supraspinal and spinal respiratory activity. Zimmer and Goshgarian (43) demonstrated that cervical spinal cord injury induced a reduction in respiratory frequency, a blunted response to pH, and changes in medullar receptor expression in neonatal rats. Golder et al. (13) observed a reduction in the phrenic burst frequency of anesthetized, vagotomized, and ventilated C₄Hₓ rats at 2 mo, but not 1 mo, postinjury during the normocapnic baseline condition. In addition, our laboratory’s previous report demonstrated that the inhibitory effects of positive end expired pressure (6 and 9 cmH₂O) on respiratory frequency was attenuated at 2 but not 8 wk postinjury. In other words, the respiratory frequency of cervical spinal cord injured animals is higher at 2 wk than at 8 wk postinjury as the result of an increase of positive end expired pressure (26). These studies suggest a supraspinal effect of cervical spinal cord injury on respiratory regulation, and, therefore, the gradual recovery of respiratory frequency observed in the C₄Hₓ animals may be attributed to the alteration of the central respiratory pattern.

Although the tidal volume was significantly lower in the C₄Hₓ animals than in the control animals at all time points postinjury, there was a time-dependent recovery after midcervical spinal cord injury. Several potential mechanisms may underlie a progressive increase of tidal volume in C₄Hₓ animals. First, ipsilateral phrenic motor outputs contribute to the recovery of tidal volume. The activation of ipsilateral phrenic bursting could be mediated by both ipsilateral bulbospinal pathways and contralateral crossed phrenic pathways. The phrenic motoneuron pool of adult rats is primarily located in

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**Table 5. MAP pressure and HR in control, C₂Hₓ, and C₄Hₓ animals during the baseline, vagotomized, and hypercapnic condition**

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>95 ± 3</td>
<td>454 ± 6</td>
</tr>
<tr>
<td>C₂Hₓ</td>
<td>82 ± 6</td>
<td>460 ± 6</td>
</tr>
<tr>
<td>C₄Hₓ</td>
<td>90 ± 5</td>
<td>460 ± 13</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>92 ± 4</td>
<td>452 ± 4</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>115 ± 7⁺</td>
<td>441 ± 5</td>
</tr>
<tr>
<td>102 ± 5*</td>
<td>470 ± 7</td>
<td>464 ± 6</td>
</tr>
<tr>
<td>109 ± 4*</td>
<td>467 ± 12</td>
<td>450 ± 11b</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate. *P < 0.01 compared with the value during the baseline and vagotomized condition. **P < 0.01 compared with the value during vagotomy.
the C3-C6 spinal cord (19); therefore, some phrenic motoneurons above the lesion continued to receive the ipsilateral bulbospinal pathway from the rostral ventral respiratory group (27). However, the majority of this ipsilateral respiratory pathway innervating the phrenic motoneurons below the lesion (e.g., C5 spinal cord) was interrupted after C4Hx. Therefore, the latent crossed phrenic pathway may be activated following chronic C4Hx to drive C5 phrenic motoneurons and improve tidal volume recovery. Second, compensatory increases in the contralateral (i.e., uninjured side) phrenic motor outputs could be evoked to maintain essential ventilation. Miyata et al. (32) demonstrated that the contralateral hemidiaphragm activity was increased after unilateral cervical spinal hemisection. The present result also demonstrated that the contralateral phrenic burst amplitude of the C4Hx animals was greater than that of control animals, suggesting that C4Hx animals utilize a higher capacity of contralateral phrenic motor output. Third, the compensatory plasticity of the nonphrenic motor system may enable tidal volume to gradually recover. Lane et al. (22) observed that breathing patterns were not significantly affected after midcervical midline contusion, despite the impairment of the hypercapnic response of the diaphragm EMG activity. Our laboratory’s recent study showed that the ipsilateral intercostal activity was abolished at 1–3 days post-C2Hx, but could be returned to the control value at 2 wk postinjury. A linear regression analysis indicated that the recovery of tidal volume is significantly correlated with ipsilateral intercostal EMG activity following high-cervical spinal cord injury (9). Accordingly, activation of the intercostal muscles may also contribute to the gradual recovery of tidal volume following C4Hx.

Comparison of Respiratory Motor Output Following High- and Midcervical Spinal Cord Injury

Although high- and midcervical spinal cord hemisection induced a similar rapid, shallow breathing pattern at 1 day postinjury, the C2Hx and C4Hx animals showed a differential recovery progress in respiratory behaviors after 1 wk postinjury. Our results demonstrated that the respiratory frequency of C4Hx animals gradually returned to the normal value; however, C2Hx animals still maintained a rapid breathing pattern. In addition, the tidal volume was gradually recovered at 1 wk postinjury in the C4Hx animals, but the tidal volume of the C2Hx animals only showed significant improvement at 4 wk postinjury. These results suggest that a rapid, shallow breathing pattern was induced by cervical spinal cord hemisection, regardless of whether the injury was a high- or midcervical injury. Nevertheless, the respiratory recovery progress after 1 wk postinjury is dependent on the injury level. C2Hx surgery interrupted the majority of the ipsilateral bulbospinal respiratory pathway; therefore, the respiratory recovery of the C2Hx animals mainly resulted from the activation of the crossed spinal respiratory pathways to the phrenic and/or intercostal motoneurons (8, 9, 15). The crossed phrenic pathway in the C4Hx animals may not have been able to trigger all phrenic motoneurons, because a proportion of the phrenic motoneuron pool was removed after hemisection surgery. Thus the activation of the spared ipsilateral phrenic motoneuron (e.g., C3 phrenic motoneuron) and/or uninjured contralateral phrenic motoneurons should be involved in the recovery of tidal volume in C4Hx animals. Although the tidal volume recovery was greater in the C4Hx animals than the C2Hx animals during baseline breathing, the time-dependent recovery of tidal volume was similar between them during hypercapnia. In other words, the capacity to increase tidal volume during respiratory challenge was blunted in the C4Hx animals. Similar results showing a blunted hypercapnic response was also observed in spinal cord injured patients (29).

The neurophysiological data demonstrated that the ipsilateral phrenic motor output in the C4Hx animals maintained a lower baseline activity, but reserved a greater capacity to increase its activity in response to respiratory challenge, while the C4Hx animals utilized a higher baseline activity, but had a weaker response during high respiratory drives. These results suggest that, although high- and midcervical spinal hemisection both reduced the ipsilateral phrenic burst amplitude, the neuroplasticity of phrenic motor output is differentially expressed in C4Hx vs. C2Hx animals. Future studies that specifically investigate changes in respiratory neuroplasticity following high- vs. midcervical spinal cord injury are warranted.

REFERENCES


