Recovery of phrenic activity and ventilation after cervical spinal hemisection in rats

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Fuller, David D., Francis J. Golder, E. B. Olson Jr., and Gordon S. Mitchell. Recovery of phrenic activity and ventilation after cervical spinal hemisection in rats. J Appl Physiol 100: 800–806, 2006. First published November 3, 2005; doi:10.1152/japplphysiol.00960.2005.—We tested two hypotheses: 1) that the spontaneous enhancement of phrenic motor output below a C2 spinal hemisection (C2HS) is associated with plasticity in ventrolateral spinal inputs to phrenic motoneurons; and 2) that phrenic motor recovery in anesthetized rats after C2HS correlates with increased capacity to generate inspiratory volume during hypercapnia in unanesthetized rats. At 2 and 4 wk post-C2HS, ipsilateral phrenic nerve activity was recorded in anesthetized, paralyzed, vagotomized, and ventilated rats. Electrical stimulation of the ventrolateral funiculus contralateral to C2HS was used to activate crossed spinal synaptic pathways phrenic motoneurons. Inspiratory phrenic burst amplitudes ipsilateral to C2HS were larger in the 4- vs. 2-wk groups (P < 0.05); however, no differences in spinally evoked compound phrenic action potentials could be detected. In unanesthetized rats, inspiratory volume and frequency were quantified using barometric plethysmography at inspired CO2 fractions between 0.0 and 0.07 (inspired O2 fraction 0.21, balance N2) before and 2, 3, and 5 wk post-C2HS. Inspiratory volume was diminished, and frequency enhanced, at 0.0 inspired CO2 fraction (P < 0.05) 2-wk post-C2HS; further changes were not observed in the 3- and 5-wk groups. Inspiratory frequency during hypercapnia was unaffected by C2HS. Hypercapnic inspiratory volumes were similarly attenuated at all time points post-C2HS (P < 0.05), thereby decreasing hypercapnic minute ventilation (P < 0.05). Thus increases in ipsilateral phrenic activity during 4 wk post-C2HS have little impact on the capacity to generate inspiratory volume in unanesthetized rats. Enhanced crossed phrenic activity post-C2HS may reflect plasticity associated with spinal axons not activated by our ventrolateral spinal stimulation.

A small percentage of respiratory bulbospinal projections to phrenic motoneurons cross the spinal midline caudal to C2 (the “crossed phrenic pathway”; Ref. 18). These crossed phrenic pathways are ineffective immediately after an acute C2 spinal hemisection (C2HS), but they can be recruited by increasing respiratory drive (18). However, with time after C2HS, the efficacy of crossed spinal synaptic pathways to phrenic motoneurons increases, partially restoring phrenic nerve and hemidiaphragm function during normoxic, normocapnic breathing (10, 14, 16, 24).

Plasticity within the spinal cord, and in particular within the phrenic motor nucleus, may underlie the increased efficacy of crossed spinal synaptic pathways to phrenic motoneurons after chronic C2HS (18, 23). For example, rapid (e.g., hours to days) morphological changes occur in the ipsilateral phrenic motor nucleus after C2HS, including an increase in the number of dendrodendritic appositions and synaptic active zones and a decrease in the somatic surface area of phrenic motoneurons (19, 22, 30). These morphological changes have the potential to increase spinal synaptic efficacy and/or phrenic motoneuron excitability and, therefore, the strength of crossed spinal synaptic pathways to phrenic motoneurons. Within the cervical spinal cord, descending projections to phrenic motoneurons travel in the dorsal or ventral aspects of the lateral funiculus (21), and evoked phrenic potentials can be elicited by electrical stimulation in either of these tracts (20). Our laboratory previously reported that plasticity in ventrolateral cervical spinal pathways correlates with ipsilateral phrenic motor recovery after C2HS (9, 10). However, in those studies, phrenic motor recovery was accelerated by a preconditioning lesion (cervical dorsal rhizotomy; Ref. 9) or by postinjury exposure to chronic intermittent hypoxia (10). Whether or not the same ventrolateral spinal synaptic pathways are associated with naturally occurring increases in crossed phrenic activity after C2HS is unknown. Accordingly, we hypothesized that spontaneous recovery of crossed phrenic motor output after chronic C2HS reflects plasticity in ventrolateral crossed spinal synaptic pathways to phrenic motoneurons ipsilateral to injury. More specifically, we hypothesized that electrical stimulation of the cervical ventrolateral funiculus contralateral to C2HS would reveal the following: 1) decreased threshold current necessary to evoke compound action potentials in the contralateral phrenic nerve; and 2) increased evoked potential amplitudes between 2 and 4 wk post-C2HS.

Cervical hemisection and phrenic motor recovery are used widely as a model for spinal cord injury research (9, 10, 12, 25, 26, 28, 29). However, the persistent (e.g., >1 wk) impact of C2HS on the pattern of breathing in unanesthetized, unrestrained animals is unknown. The time-dependent, spontaneous appearance of crossed phrenic inspiratory activity after C2HS suggests that the predictable effects of this injury on the pattern of breathing (e.g., diminished inspiratory volume, increased breathing frequency; Ref. 13) are not static, but they may change with time postinjury. Because there is a strong correlation between the amplitude of integrated inspiratory phrenic activity and the inspiratory pressure generated in spontaneously breathing animals (7), time-dependent increases in crossed spinal synaptic pathways to phrenic motoneurons (10, 14, 16, 24) may be functionally significant in terms of the capacity to generate inspiratory effort. Specifically, enhanced crossed phrenic activity may increase inspiratory pressure, thus increasing inspiratory tidal volumes. However, crossed phrenic pathways appear to make little, if any, contribution to inspiratory volume during quiet breathing (15). The functional role of...
crossed phrenic pathways may be greater under conditions of elevated inspiratory drive (15). Accordingly, we used barometric plethysmography to determine the impact of chronic C2HS on ventilation and the pattern of breathing in unanesthetized rats breathing normoxic and hypercapnic mixtures. We hypothesized that C2HS-induced deficits in the capacity to increase inspiratory volume during hypercapnia would spontaneously improve over several weeks postinjury.

METHODS

Experiments were conducted on male Sprague-Dawley rats (n = 20, 3–5 mo of age) obtained from Charles River Laboratories (colony 217, Kingston, NY). Rats were housed individually with free access to food and water. All procedures were approved by the University of Wisconsin, School of Veterinary Medicine Animal Care and Use Committee.

Recovery surgery (before neurophysiology and plethysmography). Before C2HS, rats were anesthetized with isoflurane in a closed chamber. Rats were either orotracheally intubated and ventilated with 2–3% isoflurane (balance O2) or breathed the same mixture spontaneously through a nose cone. All rats received an analgesic (buprenorphine, 0.1 mg/kg), an anti-inflammatory drug (carprofen, 4 mg/kg), and an antibiotic (enrofloxacin, 5 mg/kg). Intubated rats received the sedative medetomidine (100 µg/kg) before isoflurane to facilitate the procedure. Medetomidine was actively reversed with atipamezole (500 µg/kg) after surgery. The cervical spinal cord was exposed with a dorsal approach (C2 laminectomy and durotomy), and the spinal cord procedure. Meditomidine was actively reversed with atipamezole (500 µg/kg) after surgery. The cervical spinal cord was exposed with a dorsal approach (C2 laminectomy and durotomy), and the spinal cord was hemisected caudal to the C2 dorsal roots with microscissors. The hemisection was visually confirmed by creating an ~1-mm gap in the spinal cord using a blunt-tipped 25-gauge needle connected to a suction pump. Wounds were sutured, and the skin was closed with wound clips.

At least 1 wk before the first plethysmography experiment, a temperature telemetry transmitter (Mini-Mitter, Sunriver, OR) was surgically placed in the peritoneal cavity. Isoflurane anesthesia was maintained (2–3%) as described above. Carprofen (Rimadyl injectable, 4 mg/kg sc) was administered before surgery. A laparotomy, consisting of a small midline incision (1–1.5 cm), was performed to enable placement of the transmitter in the peritoneal cavity. The wound was sutured, and the skin was closed with wound clips. Rats received buprenorphine (0.1 mg/kg ip) before termination of isoflurane anesthesia.

Neurophysiology (unanesthetized rats). Terminal neurophysiology experiments were conducted at 2 wk (n = 7) and 4 wk (n = 8) post-C2HS. Isoflurane anesthesia was induced in a closed chamber and maintained (2–3%) via nose cone. Rats were then tracheotomized, vagotomized, and mechanically ventilated throughout the remainder of the experiment. The femoral vein was catheterized, and rats were then exposed to 5-min period of hypercapnia (PETCO2 ~ 80 Torr) by raising the inspired CO2 content.

Plethysmography (unanesthetized rats). Flow-through, barometric plethysmography was used to quantify ventilation in awake rats as described previously (27). Using a repeated-measures design, five rats were studied before C2HS and again at 2, 3 and 5 wk post-C2HS. Rats were placed in a Plexiglas chamber, and body temperature was maintained (2–3%) as described above. Carprofen (Rimadyl injectable, 4 mg/kg sc) was administered before surgery. A laparotomy, consisting of a small midline incision (1–1.5 cm), was performed to enable placement of the transmitter in the peritoneal cavity. The wound was sutured, and the skin was closed with wound clips. Rats received buprenorphine (0.1 mg/kg ip) before termination of isoflurane anesthesia.

Data analyses. Amplitudes of siphonally evoked phrenic compound action potentials were quantified as \( Q \) absolute voltage (i.e., arbitrary units), 2) relative to the amplitude in the contralateral nerve, and 3) relative to the amplitude at the following stimulus currents: threshold current, threshold current + 100 µA, and 1,000 µA (i.e., maximum) (9, 10). Latency was measured from the onset of the stimulus artifact to the peak of the evoked potential. Spontaneous inspiratory phrenic nerve activity was averaged over a stable 30-s period, 30–60 min after the establishment of the CO2 apneic threshold. During baseline conditions, the peak amplitudes of moving time-averaged, spontaneous inspiratory phrenic bursts were quantified as an absolute voltage (i.e., arbitrary units), as a percentage of the amplitude in the contralateral nerve, and as a percentage of the maximum amplitude observed during hypercapnia. During the hypercapnic period, the increase in phrenic burst amplitude was quantified as an absolute voltage, as a percent increase from baseline burst amplitude, and as a percentage of the amplitude in the contralateral nerve.

Plethysmography data were initially analyzed in 1-min bins. For the baseline, normoxia, and normocapnia condition, data represent the average of 10 consecutive 1-min bins just before hypercapnia. For hypercapnia, we report the peak tidal volume, frequency, and minute ventilation occurring over the course of each 10-min exposure.

Statistical analyses were performed using commercially available software (SigmaStat, SPSS, Chicago, IL). In cases where variables had comparable units for both ipsilateral and contralateral nerve activity (e.g., µA, V), data were compared using two-way analysis of variance and the Student-Neuman-Keuls post hoc test. Variables for which ipsilateral and contralateral nerve data could not be directly...
compared (e.g., ipsilateral phrenic amplitude expressed as a percentage of the contralateral amplitude) were analyzed using an unpaired $t$-test contrasting the 2- vs. 4-wk post-C2HS response. Comparisons of arterial blood gases, $CO_2$ apneic threshold, and mean arterial pressure were also made using an unpaired $t$-test. Statistical significance was designated as a $P$ value of $<0.05$.

RESULTS

Anesthetized rats. Arterial blood gases during recording of phrenic inspiratory bursting were not significantly different at 2 vs. 4 wk post-C2HS. Specifically, $PaCO_2$ averaged $34 \pm 2$ and $36 \pm 2$ Torr, $PaO_2$ averaged $292 \pm 8$ and $261 \pm 16$ Torr, and pH averaged $7.419 \pm 0.026$ and $7.405 \pm 0.008$, respectively, at the 2- and 4-wk time points. The similar $PaCO_2$ values suggest that the apneic $CO_2$ threshold did not change significantly over 2–4 wk post-C2HS. In accordance, the $PETCO_2$ associated with the onset of inspiratory bursting was $34 \pm 1$ Torr at 2 wk and $34 \pm 2$ Torr at 4 wk post-C2HS. On the other hand, mean arterial pressure was significantly lower at 2 (103 ± 15 mmHg) vs. 4 wk post-C2HS (126 ± 14 mmHg; $P < 0.05$). The mean arterial pressure at 4 wk postinjury was similar to values recorded in spinally intact rats from the same colony (Golder FJ and Mitchell GS, unpublished observations).

Examples of inspiratory phrenic nerve activity are provided in Fig. 1. During baseline conditions, inspiratory burst amplitude in the phrenic nerve ipsilateral to C2HS was significantly less at 2 vs. 4 wk post-C2HS when amplitude was expressed relative to the contralateral burst (Fig. 2A; $P = 0.02$). However, the difference between burst amplitude at 2 vs. 4 wk did not achieve statistical significance when expressed as arbitrary units ($P = 0.09$) or as a percentage of the maximum burst amplitude ($P = 0.19$) (Fig. 2A). During hypercapnia, ipsilateral phrenic burst amplitude (arbitrary units) was not different between 2 and 4 wk post-C2HS (Fig. 2B). However, the increase in ipsilateral phrenic burst amplitude (% baseline) during hypercapnia was significantly larger at 2 compared with 4 wk ($P = 0.02$; Fig. 2B). This difference reflects the smaller ipsilateral burst amplitude during baseline at 2 vs. 4 wk post-C2HS (Fig. 2A). The contralateral phrenic burst amplitude during both baseline conditions and during hypercapnia was not different between the 2- and 4-wk time points, regardless of the normalization method (Fig. 2).

As expected due to C2HS, baseline phrenic burst amplitude (arbitrary units) was greater in the contralateral vs. ipsilateral

![Fig. 1. Examples of ipsilateral phrenic nerve activity recorded at 2 and 4 wk post-C2 spinal hemisection injury in anesthetized rats. Control data were obtained from a spinally intact rat, and they are presented as a contrast to the activity in the C2 spinal hemisection animals. A and B: unprocessed and moving-averaged phrenic activity, respectively, during normocapnic, hyperoxic conditions. C: examples of spinally evoked crossed phrenic potentials (stimulus current = 1,000 µA).](http://jap.physiology.org/)
respiratory parameters recorded before C2HS and 2, 3, and 5 wk post-C2HS

Table 1. Analyses of compound action potentials evoked by electrical stimulation of the ventral spinal cord contralateral to C2HS in anesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>Ipsilateral phrenic nerve</th>
<th>Contralateral phrenic nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold current, μA</td>
<td>542±53</td>
<td>104±46*</td>
</tr>
<tr>
<td>Onset latency at threshold current, ms</td>
<td>1.19±0.14</td>
<td>1.37±0.14</td>
</tr>
<tr>
<td>Threshold amplitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arbitrary units</td>
<td>0.46±0.06</td>
<td>1.37±0.14</td>
</tr>
<tr>
<td>%max</td>
<td>35.3±11.6</td>
<td>34.1±19.2</td>
</tr>
<tr>
<td>%CL</td>
<td>23.0±6.9</td>
<td>24.0±10.4</td>
</tr>
<tr>
<td>Maximum amplitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arbitrary units</td>
<td>3.78±2.02</td>
<td>21.70±10.10*</td>
</tr>
<tr>
<td>Δ %Threshold</td>
<td>755±413</td>
<td>5,085±2,361*</td>
</tr>
<tr>
<td>Δ %Threshold + 100</td>
<td>552±358</td>
<td>4,072±840*</td>
</tr>
<tr>
<td>%CL</td>
<td>45.7±14.1</td>
<td>99.1±68.4</td>
</tr>
<tr>
<td>Onset latency at maximum current, ms</td>
<td>0.94±0.09</td>
<td>0.77±0.06*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Evoked potentials were recorded in the phrenic nerve ipsilateral and contralateral to C2 spinal hemisection (C2HS). Data were recorded in separate groups of rats at 2 and 4 wk post-C2HS injury. %max, Percentage of the maximum amplitude; %CL, percentage of the contralateral phrenic amplitude at the same stimulus current; Δ %Threshold, change in amplitude expressed relative to amplitude at threshold; Δ %Threshold + 100, change in amplitude expressed relative to amplitude at a stimulus current 100 μA greater than threshold. *Different from the corresponding ipsilateral phrenic nerve data point, P < 0.05. No significant differences in any parameter were observed between 2 and 4 wk post-C2HS in either the ipsilateral or contralateral phrenic nerves.

phrenic nerve at both 2 (P = 0.01) and 4 wk post-C2HS (P = 0.01; Fig. 2A). This difference was not statistically significant, however, when amplitude was expressed as a percentage of maximum burst amplitude on the respective sides (P = 0.09; Fig. 2A). During hypercapnia, the absolute voltage recorded in the contralateral phrenic nerve was significantly larger than the corresponding ipsilateral burst at both 2 (P = 0.01) and 4 wk post-C2HS (P = 0.01; Fig. 2B). However, relative to baseline, the increase in burst amplitude during hypercapnia was actually greater in the ipsilateral vs. contralateral phrenic nerve at 2 (P = 0.01) but not 4 wk post-C2HS (P = 0.66; Fig. 2B).

Inspiratory burst frequency was not different at either time point during baseline conditions (2 wk: 44 ± 3 bursts/min, 4 wk: 47 ± 2 bursts/min; P = 0.36) or hypercapnia (2 wk: 59 ± 2 bursts/min, 4 wk: 54 ± 2 bursts/min; P = 0.20).

Representative examples of compound phrenic action potentials evoked by stimulation of the cervical ventrolateral funiculus are shown in Fig. 1. In contrast to inspiratory burst amplitude, evoked phrenic potentials were not different between 2 and 4 wk post-C2HS (Table 1). For example, the threshold stimulus current required to evoke a crossed phrenic potential was comparable at 2 vs. 4 wk (Table 1). Similarly, the amplitude of the evoked potential at any stimulus current (10−1,000 μA) was not different between time points in either phrenic nerve, regardless of the normalization procedure. The onset latency (i.e., time from stimulus artifact to the evoked potential peak) also was not different across time points in either phrenic nerve (Table 1). On the other hand, significant differences between contralateral vs. ipsilateral evoked phrenic potentials were present at both 2 and 4 wk post-C2HS (Table 1).

For example, the peak latency associated with the maximum evoked potential amplitude in the contralateral phrenic nerve was shorter than for the corresponding ipsilateral potential at both time points (P < 0.05; Table 1). Similarly, the maximum amplitude of the evoked potential was greater in the contralateral vs. ipsilateral nerve at both 2 and 4 wk, regardless of the normalization procedure (Table 1).

Unanesthetized rats. During room air breathing, minute ventilation was unaffected by C2HS (Table 2). However, the pattern of breathing was significantly altered by the injury. Inspiratory tidal volume during eucapnic normoxia decreased from 0.47 ± 0.08 (pre-C2HS) to 0.39 ± 0.03 ml/100 g at 2 wk post-C2HS. This value was not different between 2 and 5 wk post-C2HS (Table 2). Rats compensated for the reduction in tidal volume by significantly increasing respiratory frequency (Table 2). However, no changes in frequency during eucapnic normoxia were observed across 2–5 wk post-C2HS (Table 2).

Before injury, frequency and tidal volume both increased when the inspired CO2 fraction was raised from 0.0 to 0.05 (Table 3). On exposure to a inspired CO2 fraction of 0.07, tidal volume further increased, but frequency did not (Table 3). Tidal volume was significantly reduced (vs. preinjury) at both hypercapnic levels 2 wk post-C2HS (Table 3). Contrary to our original hypothesis, hypercapnic tidal volumes did not increase over the week after C2HS. Thus there were no significant differences in hypercapnic inspiratory volume between 2, 3, and 5 wk post-C2HS (Table 3). Two weeks post-C2HS, peak frequency during hypercapnia was not different from values pre-C2HS, and this value did not change over the subsequent weeks (Table 3).

Table 2. Respiratory parameters recorded before C2HS and 2, 3, and 5 wk post-C2HS during room air breathing in unanesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>Pre-C2HS</th>
<th>2 wk Post-C2HS</th>
<th>3 wk Post-C2HS</th>
<th>5 wk Post-C2HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vt, ml/min 1·100 g−1</td>
<td>42.3</td>
<td>47±5</td>
<td>44±4</td>
<td>44±3</td>
</tr>
<tr>
<td>Vt, ml/100 g</td>
<td>0.47±0.08</td>
<td>0.39±0.03*</td>
<td>0.35±0.03*</td>
<td>0.36±0.03*</td>
</tr>
<tr>
<td>Frequency, breaths/min</td>
<td>36.5</td>
<td>118±89*</td>
<td>129±11*</td>
<td>127±12*</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>38.4±0.4</td>
<td>37.9±0.4</td>
<td>38.3±0.5</td>
<td>38.2±0.5</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>357±18</td>
<td>327±19</td>
<td>346±13</td>
<td>363±17</td>
</tr>
</tbody>
</table>

Values are means ± SE. Vt, minute ventilation; Vt, inspiratory tidal volume. *Different from corresponding preinjury time point, P < 0.05.
Table 3. Respiratory parameters recorded before C2HS and 2, 3, and 5 wk post-C2HS during 2 levels of hypercapnic breathing in unanesthetized rats.

<table>
<thead>
<tr>
<th></th>
<th>Pre-C2HS</th>
<th>2 wk post-C2HS</th>
<th>3 wk post-C2HS</th>
<th>5 wk post-C2HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{E}}$peak, ml/min $^{-1}$ 100 g $^{-1}$</td>
<td>172±16</td>
<td>140±18*</td>
<td>143±19*</td>
<td>112±19*</td>
</tr>
<tr>
<td>$V_{\text{E}}$peak, ml/100 g</td>
<td>0.74±0.03</td>
<td>0.66±0.05*</td>
<td>0.68±0.09*</td>
<td>0.59±0.03*</td>
</tr>
<tr>
<td>Frequencypeak, breaths/min</td>
<td>247±22</td>
<td>239±18</td>
<td>232±10</td>
<td>192±22*</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>38.0±0.4</td>
<td>37.8±0.4</td>
<td>38.1±0.6</td>
<td>38.0±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rats were exposed to inspired $\text{CO}_2$ concentrations of 5% and 7%. $V_{\text{E}}$peak, peak minute ventilation; $V_{\text{E}}$peak, peak inspiratory tidal volume; Frequencypeak, peak inspiratory frequency. *Different from corresponding preinjury time point, $P < 0.05$.

DISCUSSION

Deficits in the capacity to increase inspiratory volume after C2HS do not spontaneously improve between the second and fifth week postinjury. Accordingly, spontaneous time-dependent increases in crossed phrenic motor output over this interval appear to make minimal contribution to the generation of inspiratory volume in unanesthetized rats. Furthermore, over a time frame (2–4 wk post-C2HS) when crossed spinal inspiratory phrenic motor output increases (Ref. 16; present study), compound phrenic action potentials evoked by stimulation of the contralateral ventrolateral funiculus did not change. Thus time-dependent increases in crossed spinal inspiratory activity after C2HS may reflect plasticity associated with spinal axons not activated by our spinal cord stimulation paradigm (e.g., dorsal or ventromedial axons). Changes in descending motor drive are unlikely to cause increased crossed spinal phrenic activity during this period because there were no associated changes in phrenic motor output on the side contralateral to C2HS.

Time-dependent enhancement of crossed phrenic activity. Hours to days post-C2HS, ipsilateral phrenic inspiratory bursts occur only during intense chemoreceptor stimulation (e.g., asphyxia), after pharmacological treatments that increase respiratory drive, or with enhanced serotoninergic function (10, 25, 26, 33–35). After this acute period, the efficacy of crossed phrenic synaptic transmission increases over weeks to months. As a result, clear ipsilateral phrenic bursting is observed during normoxic normocapnia 4 wk post-C2HS (24). Although multiple laboratories have confirmed this response (10, 14), the time course varies markedly. In this study and two prior reports (10, 16), small but significant crossed phrenic inspiratory activity was observed at 2 wk post-C2HS during normoxic normocapnia. In contrast, Nantwi et al. (24) report that ipsilateral phrenic activity is absent during spontaneous breathing at 4 wk post-C2HS, but it becomes (relatively) robust by 16 wk. However, differences between the experimental preparations make it difficult to directly compare these studies. In our experiments, animals were paralyzed and ventilated, and the arterial $\text{CO}_2$ was regulated 2 Torr above the $\text{CO}_2$ aortic threshold. Nantwi et al. quantitatively assessed phrenic motor recovery at 16 wk post-C2HS, but the relative time course of phrenic recovery was assessed qualitatively over 4–16 wk post-C2HS in spontaneously breathing, poikilocapnic rats. Accordingly, the relative rate of phrenic recovery is difficult to compare between studies. Other confounding variables that may explain variability in crossed phrenic activity include sex, rat strain, and even substrain (e.g., rats of the same strain supplied from different breeding colonies). By similarity, a form of serotonin-dependent phrenic motor plasticity known as phrenic long-term facilitation (1, 23) is influenced by sex (5) and is differentially expressed among rat strains (4) and substrains (8). In the present and prior reports from our laboratory (10), we studied male rats (Charles River Sprague-Dawley, colonies K-62 and 217), whereas Nantwi and colleagues (24) studied female rats (Sprague-Dawley, colony not reported).

Mechanisms of spontaneous phrenic motor recovery. Rapid morphological changes on or around phrenic motoneurons after C2HS (19, 22, 30, 32) have led to the suggestion that the appearance of crossed phrenic inspiratory activity after chronic C2HS reflects spinal (vs. supraspinal) plasticity (18). For example, C2HS rapidly (within hours) increases the number of dendrodendritic appositions and synaptically active zones in the ipsilateral phrenic motor nucleus (30). By 30 days post-C2HS, the average number of “active zones” on glutamatergic terminals in the phrenic motor nucleus has increased (31). Furthermore, the surface area of ipsilateral phrenic motoneuron cell bodies decreases by 14 days post-C2HS, suggesting an increase in phrenic motoneuron excitability (22). The influence of serotonin on crossed phrenic activity also points toward a spinal mechanism. The serotonin precursor 5-hydroxytryptophan reveals or enhances spinally evoked crossed phrenic compound action potentials in acute C2HS rats (20). Furthermore, pharmacological activation of serotonin type 2A receptors, which are upregulated in the region of the phrenic motor nucleus after C2HS (11), enhances crossed spinal pathways to phrenic motoneurons (2, 3, 35). Thus a variety of C2HS-induced morphological or biochemical changes on or around phrenic motoneurons have the potential to alter crossed spinal synaptic inputs to ipsilateral phrenic motoneurons.

Contrary to our original hypothesis, we observed no evidence of spinal plasticity in the evoked, crossed phrenic pathway between 2 and 4 wk postinjury. Neither the threshold current nor the peak amplitude of phrenic compound action potentials evoked by ventrolateral spinal stimulation was affected during this period (Fig. 1, Table 1). Several potential mechanisms may reconcile this finding with the simultaneously...
occurring increase in spontaneous crossed phrenic inspiratory burst amplitude (Table 1). First, spinal plasticity may occur in a synaptic pathway that was not activated by our stimulating electrode. Such pathways exist in the dorsolateral funiculus (20) and in the medial ventral white matter (21). On the other hand, it is possible that the efficacy of spinal synaptic inputs to ipsilateral phrenic motoneurons and/or ipsilateral phrenic motoneuron excitability does not change after C2HS. In this scenario, increases in inspiratory activity would result exclusively from increases in descending synaptic drive to phrenic motoneurons (i.e., supraspinal plasticity). Such an effect is not inconsistent with prior reports (19, 22, 30, 32) because the physiological significance of the morphological changes in the phrenic motor nucleus has not been unequivocally established. Golder and colleagues (14) demonstrated that hypoglossal motor output is altered by C2HS, a finding suggestive of supraspinal plasticity after spinal hemisection. However, the absence of any enhancement in spontaneous phrenic activity on the intact (contralateral to C2HS) side of the spinal cord suggests that increased descending respiratory drive is not a major factor in the enhancement of crossed phrenic activity. One conceivable explanation for these seemingly disparate results is that increases in crossed phrenic motor output at early time points (e.g., 0–2 wk post-C2HS) may result from spinal plasticity, whereas subsequent changes may reflect altered bulbospinal drive. Indeed, rapid (i.e., hours to weeks post-C2HS) morphological changes in the phrenic motor nucleus may facilitate spinal synaptic transmission, as previously suggested (18), yet changes in spinally evoked phrenic potentials would not be observed over the time frame of the present study (i.e., 2–4 wk). Lastly, our neurophysiological techniques may have been insufficient to detect spinal plasticity associated with enhanced phrenic motor output. Electrical stimulation of inputs to phrenic motoneurons during spinal stimulation may have obscured facilitatory synaptic inputs to phrenic motoneurons. Nevertheless, strong correlations between spinally evoked phrenic potentials and inspiratory phrenic motor output have been reported previously (9, 10).

**Ventilation after C2HS.** Twenty-four hours after C2HS, awake rats breathe with an elevated frequency with uncertain changes in volume (17). Anesthetized rats breathe with increased frequency and decreased tidal volume at 1 and 2 mo post-C2HS (15). Arterial blood gases measured in awake rats 24 h postinjury and anesthetized rats after chronic C2HS (1–2 mo) indicate adequate alveolar ventilation (13, 17), although rats may hypventilate during the first few hours postinjury (Fuller DD, Golder FJ, and Mitchell GS, unpublished observations). Here, we document a persistent change in the resting respiratory rate in awake rats breathing with an elevated frequency with uncertain changes in tidal volume (Table 1). First, spinal plasticity may occur in a synaptic pathway that was not activated by our stimulating electrode. Such pathways exist in the dorsolateral funiculus (20) and in the medial ventral white matter (21). On the other hand, it is possible that the efficacy of spinal synaptic inputs to ipsilateral phrenic motoneurons and/or ipsilateral phrenic motoneuron excitability does not change after C2HS.

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**References**


