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Brief Communication

Cough following low thoracic hemisection in the cat

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ABSTRACT

A function of the abdominal expiratory muscles is the generation of cough, a critical respiratory defense mechanism that is often disrupted following spinal cord injury. We assessed the effects of a lateral T9/10 hemisection on cough production at 4, 13 and 21 weeks post-injury in cats receiving extensive locomotor training. The magnitudes of esophageal pressure as well as of bilateral rectus abdominis electromyogram activity during cough were not significantly different from pre-injury values at all time points evaluated. The results show that despite considerable interruption of the descending pre-motor drive from the brainstem to the expiratory motoneuron pools, the cough motor system shows a significant function by 4 weeks following incomplete thoracic injury.

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Introduction

The expulsive component of cough is generated primarily by the coordinated activity of the anterolateral abdominal muscles (rectus abdominis, transversus abdominis, external oblique, and internal oblique). Input from the brainstem cough neural networks (for review, see Iscoe, 1998) to the motoneuron pools for these primary expiratory muscles is disrupted following cervical and thoracic injuries involving the ventral and ventrolateral spinal cord (Davis and Plum, 1972). In the cat, the motoneuron pools for these four muscles all terminate at L3, but they have varying rostral extents with the rectus abdominis extending most rostrally (T4); the external oblique to T6; the transverses abdominis to T9; and the internal oblique to T13 (Miller, 1987). Furthermore, from T1-L3, expiratory axons originating in the brainstem arborize expansively throughout the contralateral side of the spinal cord, creating an extensive network spanning several spinal segments (Merrill, 1974).

In the present study lateral T9/10 hemisections were made in adult cats. These lesions transect the descending brainstem expiratory pathways on one side of the spinal cord, disrupting pre-motor drive to the caudal, ipsilateral expiratory motoneuron pools. Cough pressure generation and rectus abdominis muscle activity were characterized pre- and post-injury. Expiratory muscle recordings were made from the rectus abdominis, because it contributes to the generation of cough expulsive forces in the cat (Tomori and Widdicombe, 1969), plays a significant role in increasing abdominal cavity pressure during cough (Bolser et al., 2000), and is easily accessed for repeated assessments. Based upon extensive ipsi- and contra-lateral arborizations of descending expiratory axons as well as expiratory associated thoracic interneurons with crossed axons spanning multiple segments (Kirkwood et al., 1988), we hypothesized that features of the cough reflex would recover following a T9/10 lateral hemisection. Our findings support this hypothesis and show that, despite considerable disruption of descending pre-motor drive from the brainstem to motoneuron pools of the primary expiratory muscles, the cough motor system shows substantial function by 4 weeks (earliest time point studied) following thoracic spinal cord injury (SCI).

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Methods

Cough production was assessed pre- and post-spinal T9/10 left hemisection in six specific-pathogen-free adult, spayed female cats (6–8 lbs, Liberty Laboratories, NY). Hemisections and post-op care were performed as described previously (Tester and Howland, 2008). Surgeries were performed under isoflurane anesthesia. Buprenorphine (0.02 mg/kg) was given TID for 48 h, and bladders expressed manually for 1–5 days, post-SCI. Cat body weights were closely monitored post-injury and were maintained within 5% (+/-) of their pre-injury weights. Cats were housed on thick cushions in the AALAC accredited animal facility and trained on a variety of locomotor tasks 5 times/week) for a parallel study. Animal procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the University of Florida's IACUC.

Cough was assessed in isoflurane anesthetized, spontaneously breathing cats pre-injury and at 4, 13 and 21 weeks post-SCI. Atropine sulfate (0.1 mg/kg, SQ) was given to block salivation and tracheal secretions. End-tidal CO_2 was monitored and isoflurane levels adjusted to maintain this parameter within 4–5%. Cats were placed in the supine position, and a sterile abdominal field prepared. Paired bipolar Teflon-coated stainless steel wire electrodes were placed 2–3 mm apart in the left and right rectus abdominis muscles approximately 2 cm caudal to the iliac crest and 1 cm lateral to midline. A ground electrode was placed in the left hamstring. An esophageal balloon catheter was placed at the midthoracic level and cough elicited by mechanical stimulation of the vocal folds and epiglottis using an oral approach and a small length of flexible plastic tubing (Bolser, 1991; Bolser et al., 1993). Isoflurane was administered via a nose cone. Once animals reached a surgical plane of anesthesia, determined by lack of reflex responses to whisker stimulation, paw web pinch or cutaneous stimulation of the eye, the nose cone was removed and cough was mechanically elicited. Once the cough bout ended, the nose cone was replaced and the sequence repeated with the goal of collecting approximately 20 coughs for each cat. Following cough stimulation, animals were recovered and monitored for the next few hours to verify that they resumed eating and drinking.

Esophageal pressure (Pes) and left and right rectus abdominis (LRA and RRA) electromyograms (EMGs) were recorded. Pes (cmH_2O) was used as it has been shown to reflect abdominal pressure (Bolser et al., 2000). The esophageal balloon catheter was calibrated prior to each experiment and placed in the midthoracic esophagus. The pressure response of the balloon catheter system was linear over the pressure range of coughing (0–120 mm Hg). EMGs were amplified and digitally band-pass filtered (200–5000 Hz). The EMGs were rectified and moving averages (sliding window 50 ms) of these signals were generated off-line using a custom script employing Spike2 software (Cambridge Electronic Design, Inc.). Coughs were identified by behavioral observation of the animal and the presence of Pes amplitude larger than 5 cm H_2O in response to the mechanical stimulus. Six parameters were calculated: Pes, percentage of LRA and RRA normalized cough amplitudes, esophageal rise times, and LRA and RRA rise times. To obtain RA EMG normalized amplitudes, each cough amplitude was expressed as a percentage of the maximal burst at a given time point for each side in each cat. These normalized percentages were averaged for each cat at each time point. Statistical analyses were run on these normalized (percent) values of EMG magnitude. Rise times were determined as the elapsed time between 10% and 90% of the total rise time of the moving average. Individual cough rise times for each cat were averaged at each time point.

Using SPSS software 14.0 (Chicago, IL), separate repeated measures, within-subjects ANOVAs were conducted to determine if esophageal parameters (pressure and rise time) differed across time points. Mixed (time × side) two-factor ANOVAs were conducted to determine if there was an effect of time or side on rectus abdominis EMG rise times and normalized percentage of amplitudes. Post-hoc Fishers LSD tests were used to isolate any differences identified with ANOVAs. Spearman correlation coefficients were conducted to determine if any variation in lesion size was associated with any of the behavioral parameters assessed. An α level of 0.05 was used for all analyses. Data are shown as averages ± standard deviations.

Following the last cough data collection at 21 weeks post-SCI, cats were deeply anesthetized (sodium pentobarbital, >35 mg/kg I.P.) and transcardially perfused with 0.9% saline (200–400 ml) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (3.5 l, pH 7.4). Spinal cord tissue was removed, blocked, cryoprotected in 30% sucrose-paraformaldehyde, and sectioned at 25 µm on a cryostat. Four of the cats' lesions were cut coronally (axially) and the remaining two cut longitudinally. One section of every ten was mounted onto chrom alum and poly-L-lysine-coated slides (chromium potassium sulfate and poly-L-lysine, Sigma-Aldrich, St. Louis, MO; gelatin, Fisher Scientific, Hampton, NH) and processed with cresyl violet (cresyl violet with acetate, Sigma Aldrich, St. Louis, MO) and myelin (Eriochrome Cyanine R, Fluka, New York, NY) stains for basic histology to determine the extent of injury following procedures detailed previously (Tester and Howland, 2008). The damage for each animal at the lesion epicenter was determined by microscopic examination of cresyl violet and myelin stained sections throughout the lesion site and represented in cross section. For four of the cats, the lesion areas were directly outlined from cross sections of tissue (Figs. 1A, B, C, and E). For the remaining two cats, whose tissue had been cut longitudinally, the representations were produced by examining multiple longitudinal sections to appreciate the cross-sectional extent of the lesions, which were then imposed upon a representative cross sectional drawing (Figs. 1D, F).

Results

A total of 256 coughs from six cats were analyzed across four time points: pre-injury ($n = 37$); 4 weeks post-hemisection (wphx, $n = 87$); 13 wphx ($n = 75$); and 21 wphx ($n = 57$). Cresyl violet and myelin stained serial sections of each lesion were assessed to determine the extent of SCIs. The lesions ranged from under-hemisections with partial ipsilateral medial-ventral white matter sparing (Figs. 1A, B) to a complete hemisection (Fig. 1C) to over-hemisections with variable disruption of contralateral gray and white matter in the dorsal or ventral aspect of the spinal cords (Fig. 1D, E, F). Spearman correlation coefficients showed no significant relationship between lesion size and behavioral parameters, suggesting that the expiratory pressures and bilateral RA EMGs features assessed in this study were not influenced by these injury differences.

All cats generated coughs pre- and post-injury under anesthesia in response to mechanical stimulation of the epiglottis and vocal folds. As assessed by EMGs, the RA muscles were normally silent during eupneic breathing. RA EMGs and Pes increased during mechanically elicited cough. Individual coughs as well as repetitive cough bouts were frequently generated after injury (Fig. 2A). Pes and RA EMGs during coughing were similar to pre-injury recordings. The EMG patterns at all post-injury time points were typical of ballistic-like bursting observed in uninjured animals (Bolser et al., 2000) (Fig. 2B). Moreover, they were present bilaterally. Finally, the peak EMG activities of the LRA and RRA during cough occurred simultaneously and were correlated with increases in Pes. Thus, qualitatively, these general cough characteristics appeared similar to those observed prior to injury. Quantitative assessments below support these observations.

Cough Pes, measured in cm H_2O, at each time point were: 64.06 ± 39.75 (pre-SCI), 58.38 ± 32.00 (4 wphx), 39.94 ± 25.91 (13 wphx) and 68.93 ± 38.51 (21 wphx). No significant change in the average Pes was seen across time points ($p = 0.410$). In addition, during some individual coughs post-operatively, Pes reached or exceeded 100 cm H_2O indicating that the injured system was capable of generating the substantial pressures sometimes seen pre-injury, as well as in other