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Influence of intrathoracic vagotomy on the cough reflex in the anesthetized cat

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Abstract

Recurrent laryngeal afferent fibers are primarily responsible for cough in response to mechanical or chemical stimulation of the upper trachea and larynx in the guinea pig. Lower airway slowly adapting receptors have been proposed to have a permissive effect on the cough reflex. We hypothesized that vagotomy below the recurrent laryngeal nerve branch would depress mechanically or chemically induced cough. In anesthetized, bilaterally thoracotomized, artificially ventilated cats, thoracic vagotomy nearly eliminated cough induced by mechanical stimulation of the intrathoracic airway, significantly depressed mechanically stimulated laryngeal cough, and eliminated capsaicin-induced cough. These results support an important role of lower airway sensory feedback in the production of tracheobronchial and laryngeal cough in the cat. Further, at least some of this feedback is due to excitation from pulmonary volume-sensitive sensory receptors.

Keywords

Cough; Recurrent laryngeal afferent; Pulmonary stretch receptor

1. Introduction

The cough reflex is essential in the clearance of foreign material, infectious agents, and mucus from the airways. The consequences of poor airway clearance can include pulmonary

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Author contributions

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Declaration of Competing Interest

None.

infection, chronic inflammation, and long-term damage to the lung airways or parenchyma (Leith et al., 2011; Stjärne Aspelund et al., 2018). The cough reflex in both human subjects and animals can be initiated through mechanical and chemical stimulation of the upper airways and the tracheobronchial tree (Canning et al., 2006; Karlsson and Fuller, 1999; Laude et al., 1993; Mazzone and Undem, 2016; Tatar et al., 1994; Widdicombe, 1996).

Vagal afferents in cat, dog, rabbit and guinea pig are known to be important in evoking cough in response to mechanical and chemical stimuli (Cinelli et al., 2012; Haná ek et al., 1984; Tatar et al., 1994, Canning et al., 2006 #3; Widdicombe, 1954a, 1998; Widdicombe, 1996). Cervical vagotomy abolishes the cough reflex in response to mechanical stimulation (Canning et al., 2004; Klassen et al., 1951; Tatar et al., 1988; Widdicombe, 1954a), and cervical vagal cooling has been shown to reversibly decrease the cough reflex (Hanacek, 1987; Simera et al., 2016). Pulmonary slowly adapting receptors (SARs), rapidly adapting receptors (RARs), and pulmonary C-fibers all have been proposed to influence the production of cough (Karlsson et al., 1988). The recent discovery of specific cough receptors has led to uncertainty regarding the role of RARs on the production of cough (Canning et al., 2004, 2006). Lower airway C-fibers have been functionally separated into cough excitatory and cough inhibitory populations (Canning et al., 2006). Evidence for an excitatory population of C-fibers on cough has been derived primarily from the guinea pig model (Canning et al., 2006). Inhibitory effects of airway C-fiber stimulation have been observed in the cat and dog (Simera et al., 2016; Tatar et al., 1994, 1988) and in the guinea pig (Chou et al., 2018). C-fibers originating from the nodose ganglia were inhibitory for cough, whereas C-fibers originating from the jugular ganglia were found to be key in initiating and/or sensitizing the cough reflex (Chou et al., 2018). The effect of SARs on cough has been proposed to be permissive for coughing due to lower airway afferents (Haná ek et al., 1984; Sant' Ambrogio et al., 1984; Tatar et al., 1994, 1988) and facilitatory for cough elicited by chemical stimulation of the larynx (Haná ek et al., 1984), but not directly involved in evoking cough (Mazzone and Undem, 2016).

The role of SARs in cough production has been questioned recently based, in part, on the nonspecificity of sulfur dioxide (Canning et al., 2006), which was employed in these previous studies to block SARs in the rabbit (Haná ek et al., 1984; Sant' Ambrogio et al., 1984); sulfur dioxide can excite airway C-fibers (Atzori et al., 1992; Lin et al., 2020; Wang et al., 1996). However, recent work by Poliaček and coworkers (2016) (Poliaček et al., 2016) has shown that the cough motor pattern can be altered by out-of-phase inflations delivered by a custom mechanical ventilator in animals with intact chest walls. The authors concluded that the cough motor pattern was modified in their study by volume-related feedback in a manner consistent with that of breathing (Hering and Breuer, 1868). However, these investigators did not separate volume-related feedback from sensory afferents in the lungs and airway from the chest wall. As such, the role of lower airway SARs in the production of cough remains unresolved but may play an important role in cough production.

The recurrent laryngeal nerve (RLN) branches from the cervical vagus within the thorax and innervates the extrathoracic trachea and larynx. In the guinea pig, the RLN has been shown to be critical for the cough response to mechanical perturbations in the trachea and larynx (Canning et al., 2004; Tsubone et al., 1991; Tsujimura et al., 2013). We hypothesized that

acute bilateral transection of the intrathoracic vagus nerves distal to the branch of the RLN would not extinguish mechanically- or chemically-stimulated tracheobronchial (TB) cough or mechanically-stimulated laryngeal (LAR) cough, but would decrease cough excitability due to loss of lower airway mechanoreceptors.

2. Materials and methods

2.1. Animals

Experiments were performed on 22 adult American Shorthair cats (16 male, 6 female, weight range 3.2 kg–6.3 kg, 27–54 months, Marshall BioResources, North Rose, New York).

2.2. Ethics statement

The experimental protocol was approved by the University of Florida Institutional Animal Care and Use Committee (IACUC), and all procedures are compliant with the Guide for the Care and Use of Laboratory Animals. All efforts were taken to minimize animal suffering and to reduce the number of animals used.

2.3. Surgical preparation

Twenty-two cats were anesthetized initially with sevoflurane gas (3–5 %) (Abbott Labs, Chicago, IL) followed by sodium pentobarbital (25 mg/kg, i.v., Akorn Pharmaceuticals, Lake Forest, IL). After sodium pentobarbital was administered, sevoflurane was discontinued. Anesthetic depth was assessed every 15 min by checking for the presence of corneal and forelimb pull back reflex, jaw tone, and reflexive changes in respiratory rate and/or blood pressure. When necessary, supplemental doses of sodium pentobarbital were administered as needed (1–3 mg/kg, i.v.). A dose of atropine sulfate (0.053 mg/kg, i.v., Patterson Veterinary Supply, Inc., Devens, MA) was administered at the beginning of the experiment to reduce secretions from repeated perturbation of the inner trachea and larynx. At the end of the experiment, the cats were euthanized with an overdose of anesthesia (i.v.) followed by a saturated potassium chloride solution (3.0 mL, i.v., Fisher Scientific, Waltham, MA).

Cannulas were placed in the trachea (at the level of the tenth tracheal ring), femoral artery, and femoral vein. A portion of the lateral cartilage of the 10th tracheal ring was removed to allow easier access to stimulate the larynx. To measure intrathoracic pressure in animals with closed chests (pre-thoracotomy), an esophageal balloon attached to a pressure transducer was placed by an oral approach. After thoracotomy, a pressure transducer was attached to the tracheotomy port to measure tracheal airflow. End-tidal CO₂ from the tracheotomy port (GEMINI O₂ & CO₂ Monitor, CWE Inc., Ardmore, PA) and femoral arterial blood pressure (CED 1902, Oxford, UK) were monitored continuously. Rectal temperature was monitored continuously and maintained at 37.5 ± 0.5 °C using a homeothermic pad (TC-1000, CWE Inc., Ardmore, PA). Arterial blood was sampled periodically to measure blood gases (Element POC, Heska, Loveland, CO).

Thoracotomies were performed via an incision at the 2nd intercostal space on the right side of the animal and the 4th intercostal space on the left and extended from sternum to axilla. The animal was artificially ventilated at a rate and tidal volume (approximately 25 mL) sufficient to maintain normal blood gasses (pH 7.39 ± 0.01 ; PaO₂ 100.35 ± 1.66 mmHg; PaCO₂ 34.96 ± 0.72 mmHg).

Positive end-expiratory pressure (PEEP) was maintained at 5 cmH₂O. In a subset of these animals (n = 9) a PEEP protocol was performed, where PEEP was varied between 5, 2 and 0 cmH₂O with recovery periods (5 cmH₂O PEEP) in between. Cough number did not recover after 0 cmH₂O PEEP, and so PEEP conditions were not fully randomized. Lungs were hyperinflated post-thoracotomy and after PEEP trials of 0 cmH₂O. In 11 animals, bilateral intrathoracic vagotomy was conducted by transecting the vagus nerve distal to the branching of the RLN. Visual identification of the branching point of the RLN from the vagus and prolongation of inspiratory duration (Karczewski and Widdicombe, 1969) after vagotomy were used to confirm the rightness of the vagotomy.

2.4. Data collection

Electromyogram (EMG) tracings were recorded with bipolar, insulated, fine wire hook electrodes. All EMG signals were acquired using the Spike2 (Version 8, Cambridge Electric Design, Inc., Cambridge, UK) software with the CED 1401 hardware. The EMG signals were amplified and filtered (0.3–10 kHz; Grass Model P511, Astro-Med, West Warwick, Rhode Island). The muscle activity of the diaphragm (Dia-inspiratory) and internal abdominal oblique muscles (Abd-expiratory) were used to determine cough motor response and pattern (Fig. 1). Dia EMG electrodes were placed by inserting the electrode at the point just inferior to the xiphoid process with the tip pointed posteriorly and cranially. The electrodes for the internal abdominal oblique muscles were placed in the anterior-lateral abdomen after displacing the exposed external abdominal oblique muscle, thereby exposing the surface of the internal abdominal oblique muscle. Other EMG electrodes were placed to identify other airway protective behaviors. These muscles include the parasternals (third intercostal space), mylohyoid, geniohyoid, thyroarytenoid, posterior cricoarytenoid, thyrohyoid, thyropharyngeus, and upper esophageal sphincter muscles. Electrodes were placed approximately 2 mm apart, and their positions were confirmed by both visual inspection and by the patterns of the EMG activities during cough, breathing and swallow. Cough was defined as increased parasternal and Dia EMG activity immediately followed by ballistic-like Abd EMG signal. We sometimes observed other airway reflexes, including expiration reflex (forced expiratory effort against a closed glottis, which is characterized by a ballistic expiratory burst and no corresponding inspiratory activity), aspiration reflex (a rapid and strong gasp-like inspiration characterized by a ballistic inspiratory activity and no corresponding expiratory activity), and swallow (characterized by coordinated pharyngeal and laryngeal muscle activity). These reflexes have unequivocally different motor patterns than cough.

2.5. Protocol

Mechanically stimulated TB cough was induced by repeatedly inserting and rotating a thin polyethylene tubing (inner diameter: 0.863 mm, outer diameter: 1.27 mm, wall thickness:

0.203 mm, A–M Systems, Sequin, WA) into the lower airways via the tracheostomy tube at about 2 Hz for 10–30 s with a 1 min recovery period. The end of the catheter was as deep as the carina. LAR cough was mechanically induced by a punctate stimulus with the same-sized polyethylene tubing used for TB cough stimulation. This cannula was inserted into the trachea via the tracheotomy incision and advanced rostrally. Capsaicin-induced cough (CAP) was elicited by injecting capsaicin solution (10 μ M capsaicin, 0.5 mL, diluted in saline from 100 mM capsaicin stock solution made up in 100 % ethanol) into the lower airways via the tracheostomy tube. The vehicle control was 100 % ethanol diluted (1–10,000 dilution) in saline.

To establish baseline cough activity, three TB cough trial stimulations between trials were performed while the animal had a closed chest. During the control condition, the thorax was open, and the vagus was intact (post-thoracotomy/pre-vagotomy - PT/PV). Control cough trials were collected as follows, with 1 min of recovery between trials: 3–4 TB trials, 2 LAR trials, 1 CAP trial. Not all animals coughed in response to all 3 types of perturbations during the control period. In that case, data for that perturbation was not included for that animal (non-responders: TB 6 of 22, LAR 14 of 20, CAP 15 of 20). The same cough trials from the control period were repeated in the post-vagotomy period. The animals were allowed to recover for a minimum of 10 min post vagotomy before initiating the first cough stimulus.

In the subset of animals where a PEEP protocol was performed, PEEP was decreased from 5 to 2 and/or from 5 to 0 cmH₂O. PEEP level was held for 3–4 min, and 2–3 TB cough trials were performed at each PEEP level.

2.6. Data analysis

Mean cough frequencies (coughs/min) were calculated for TB cough, and the mean number of coughs were calculated for LAR and CAP coughs. EMG amplitude measurements were normalized to the average amplitude of TB coughs during the pre-thoracotomy period. Inspiratory (CTI), active expiratory (CTE1), and passive expiratory (CTE2) cough phase durations were measured and averaged for each condition. CTI was defined as the duration between the onset and peak cough inspiratory activity; CTE1 was the duration between peak cough-related inspiratory activity to the end of abdominal activity; CTE2 was the duration between expulsive abdominal activity and the next cough inspiratory effort.

EMG signals were rectified and integrated (time constant 50 ms) in post-processing (Spike2). EMG magnitude (peak amplitude) was measured for each cough and the average value per stimulation type was reported. EMG magnitudes for coughs of all stimulation types were normalized to the average EMG magnitude of their respective muscle type during TB coughs in the pre-thoracotomy period (baseline) in order to allow for accurate comparison of EMG magnitude changes due to vagotomy between stimulation type.

2.7. Statistical analysis

Unpaired or paired two-sided t-tests were used to test for significance between two groups, and repeated measures analysis of variance (RM-ANOVA) with a Holm-Sidak post hoc was used to determine significance between more than two groups. Missing values in RM-ANOVA tests were interpolated (SigmaPlot 13.0, Systat Software Inc, Palo, Alto, CA).

P-values < 0.05 was the threshold for statistical significance. All results are reported as mean \pm SE. Data are represented as box and whisker plots, where the center line represents the median, and the boundaries of the box indicate the 25th and 75th percentile. The whiskers (error bars) above and below the box represent the 10th and 90th percentiles.

2.8. Data share

Data have been shared in a public repository (Pennsieve).

3. Results

Thoracotomy reduced the excitability of TB cough. Pre-thoracotomy TB cough frequencies were 35.0 ± 3.3 coughs/min and were reduced to 22.6 ± 2.4 coughs/min after thoracotomy (RM-ANOVA, $p < 0.001$). Tracheobronchial cough magnitude decreased post-thoracotomy/prevagotomy (PT/PV – control), primarily driven by a decrease in expulsive (Abd EMG) activity (67.0 ± 10.6 % baseline; paired t -test, $p < 0.01$) and not inspiratory (Dia) EMG activity (92.3 ± 9.3 % baseline, paired t -test, $p = 0.4$.) (Fig. 3). Inspiratory phase durations of TB coughs post-thoracotomy were significantly longer compared to TB coughs pre-thoracotomy, but expiratory phase durations were not significantly different between the conditions (pre-thoracotomy TB coughs: CTI – 0.86 ± 0.06 s, CTE1 – 0.28 ± 0.03 s, CTE2 – 1.03 ± 0.24 s; PT/PV TB coughs: CTI – 1.1 ± 0.12 s, CTE1 – 0.25 ± 0.03 s, CTE2 – 1.39 ± 0.53 s, paired t -test, CTI – $p = 0.05$, CTE1 – $p = 0.29$, CTE2 – $p = 0.40$).

Mechanical stimuli applied to the intrathoracic trachea, larynx, and intratracheal instillation of capsaicin all induced coughing after thoracotomy, pre-vagotomy (Fig. 1). In the PT/PV period, cough phase durations (LAR: CTI – 1.34 ± 0.48 s, CTE1 – 0.26 ± 0.06 s, CTE2 – 2.45 ± 0.32 s; CAP: CTI 1.15 ± 0.26 s, CTE1 – 0.26 ± 0.07 s, CTE2 – 1.0 ± 0.29 s, one-way ANOVA, CTI – $p = 0.80$, CTE1 – $p = 1.0$, CTE2 – $p = 0.24$) and normalized magnitudes of inspiratory EMGs (LAR: 143.5 ± 41.5 % baseline; CAP: 95.4 ± 19.6 % baseline, one-way ANOVA, $p = 0.17$) were not significantly different between the 3 different cough stimulation types, but expiratory EMG magnitudes between LAR and TB or LAR and CAP were significantly different (LAR: 146.8 ± 11.0 % baseline; CAP: 54.8 ± 12.3 % baseline, one-way ANOVA, $p = 0.002$).

Intrathoracic vagotomy significantly decreased TB cough (22.6 ± 2.4 to 0.3 ± 0.3 coughs/min, RM-ANOVA, $p < 0.001$). In 11 animals, only 1 TB cough-like effort was observed post-vagotomy. This reflex had prolonged inspiratory activity, followed by abrupt, weak, expiratory activity that produced no significant change in tracheal airflow. Capsaicin-induced coughing was eliminated post-intrathoracic vagotomy (9.75 ± 4.8 coughs/bolus to 0 coughs, paired t -test, $p < 0.001$) (Fig. 2). We were able to induce LAR cough pre-vagotomy (1.5 ± 0.5 coughs/stim, paired t -test, $p = 0.002$), but only 1 of 4 animals coughed after intrathoracic vagotomy, illustrating that coughs could be elicited after intrathoracic vagotomy. For the animal that had an LAR cough response, the cough was weaker (Dia EMG – 61.2 – 34.1 % baseline; Abd EMG – 144.0 – 65.3 % baseline), inspiratory phase timing decreased (CTI – 2.51 to 1.81 s), but expiratory phase timing increased (CTE1 – 0.18 to 0.26 s; CTE2 – 1.93 – 4.92 s).

The effects of PEEP on cough excitability ($n = 9$) post-thoracotomy, pre-vagotomy in unparalyzed animals resulted in decreased cough excitability with decreased PEEP (Fig. 4). TB cough frequency at 5 cmH₂O PEEP was 15.2 ± 2.1 coughs/min, at 2 cmH₂O PEEP it was 10.9 ± 2.0 coughs/min (RM-ANOVA, $p = 0.06$ compared to 5 cmH₂O) and at 0 cmH₂O PEEP it was 1.8 ± 1.0 coughs/min (RM-ANOVA, $p < 0.05$ compared to 2 and 5 cmH₂O PEEP groups). Only 3 of 9 animals coughed at 0 cmH₂O PEEP. When returned to 5 cm H₂O PEEP, cough frequency recovered after the 2 cm H₂O condition (9.7 ± 3.1 coughs/min, $p = 0.36$. compared to first TB trials during the first 5 cmH₂O PEEP condition), but cough frequency did not recover fully after the 0 cmH₂O PEEP condition (6.0 ± 2.4 coughs/min, $p = 0.04$). Decreasing PEEP from 5 to 2 increased cough inspiratory phase duration ($p = 0.04$), but CTI during 0 cmH₂O PEEP was not significantly different from either 5 ($p = 0.08$) or 2 cmH₂O ($p = 0.8$) PEEP conditions (CTI – 5 cmH₂O: 1.44 ± 0.21 s; 2 cmH₂O: 1.63 ± 0.21 s; 0 cmH₂O: 1.93 ± 0.32 s, RM-ANOVA). Cough expiratory phase durations were unchanged (CTE1 – 5 cmH₂O: 0.33 ± 0.05 s, 2 cmH₂O: 0.32 ± 0.06 s, 0 cmH₂O: 0.36 ± 0.10 s, $p = 0.94$; CTE2 – 5 cmH₂O: 1.34 ± 0.35 s, 2 cmH₂O: 0.82 ± 0.18 s, 0 cmH₂O: 1.05 ± 0.83 s, $p = 0.76$; RM-ANOVA). We saw no evidence of cough entrainment to the inflation phase of the ventilator. Cough EMG amplitudes also were unchanged between 5 to 2 cmH₂O PEEP conditions (Dia 57.8 ± 7.8 – 54.3 ± 5.5 % baseline, $p = 0.44$; Abd 57.1 ± 5.5 – 47.7 ± 3.9 % baseline, $p = 0.60$, RM-ANOVA).

4. Discussion

The major findings from these experiments indicate that lung vagal afferent feedback originating distal to the large airways is critical for TB and CAP cough and modulates LAR cough excitability in the cat (Fig. 2). Our data also suggest that lung volume-related feedback is important for the excitability of TB cough in the cat. Finally, we were able to induce cough in the anesthetized cat with intratracheal capsaicin.

Afferent and efferent signals through the cervical vagus and RLN are important in mechanically- and electrically-induced TB and LAR cough excitability (Canning et al., 2004; Lee et al., 2001; Tatar et al., 1994). The RLN, however, is not essential to LAR cough reflex initiation; LAR cough could be initiated when the RLN branches were severed (Canning et al., 2004) or a cold partial cervical vagal block was performed (Tatar et al., 1994). The role of vagal afferents distal to the RLN branch on cough is not known, but our results indicate that these vagal afferents are critical for LAR cough excitability. Through transecting superior laryngeal nerves (SLN) bilaterally, Canning et al. (2004) demonstrated that SLN efferent and afferent information was not required for mechanical activation of laryngeal cough, although the number of guinea pigs that coughed in response to electrically stimulating the laryngeal mucosa increased (Canning et al., 2004). Unlike for LAR cough, we observed that sectioning the vagus distal to the branch of the RLN eliminated TB and CAP coughing (Fig. 2), suggesting that vagal afferents distal to the branching point of the RLN are required for the generation of cough. Lung transplant patients have their lower airways denervated, and in these patients, laryngeal cough could be evoked 6 weeks to 36 months after surgery (Higenbottam et al., 1989), and in patients 1.5–12 weeks after surgery, cough responses were elicited from all sites except distal to the airway anastomosis (Duarte et al., 2008).

Canning (2011) observed that the intrathoracic capsaicin-induced cough is extinguished with anesthesia in the guinea pig (Canning, 2011), and another group reported similar observations in the dog (Palecek et al., 1989). We have observed that cough remains present during anesthesia in the cat when capsaicin is administered into the trachea. These apparent discrepancies may be due to a difference in species or anesthetic level. We note that Tatar et al. (1988) showed that capsaicin suppressed mechanically-induced coughing when administered by the intravenous route (Tatar et al., 1988). Our results, in combination with that of Tatar and coworkers, support the existence of multiple capsaicin-sensitive pathways in the cat that can have divergent effects on cough. This conclusion is at least partially consistent with that of Chou et al. (2018) who showed that there were multiple airway C-fiber subtypes with divergent effects on cough in the guinea pig (Chou et al., 2018). However, while our results are likely specific to airway receptors due to aerosol administration of capsaicin, those of Tatar and colleagues were not. Intravenous administration of capsaicin in their experiments could have stimulated cardiac afferents. In our experiments, intrathoracic vagotomy also severed axons from the posterior bronchial, esophageal, gastric, celiac and hepatic vagal branches. On the left side, vagal cardiac branches to the deep cardiac plexus are maintained, however, on the right side, they were severed (Fukui et al., 2019), which may diminish cardiac feedback. The role of cardiac afferent feedback in the production of cough is unknown.

Our results support the conclusion that volume-related feedback from the lower lungs results in decreased cough excitability (Fig. 4). We observed that decreasing lung inflation due to lowering PEEP decreased cough frequency but not intensity, and the dominant feature change appears to be prolonged CTI. The graded excitatory effect of PEEP suggests that decreased pulmonary stretch receptor activation is sufficient to decrease cough excitability. Changes in cough phase timing resulting from decreased PEEP are consistent with Poljacek et al. (2016), where increased lung volume during the first half of the inspiratory period of cough resulted in reduced CTI, likely due to increased slowly adapting pulmonary stretch receptor (PSR) activity earlier in the inspiratory phase of the cough (Poljacek et al., 2016). Widdicombe and coworkers (1954) previously concluded that rapidly adapting stretch receptors modulate the cough reflex and that slowly adapting stretch receptors (SARs) facilitate the cough reflex (Haná ek et al., 1984; Hanacek et al., 2006; Karlsson et al., 1988; Widdicombe, 1954b). However, our data showed that TB and CAP cough were extinguished by intrathoracic vagotomy (Fig. 2), which, in conjunction with the evidence that volume related feedback affects cough excitability (Fig. 4), are consistent with the hypothesis that lower airway SARs play a permissive role in cough excitability. In our computational modeling studies, SARs may play an indirect role due to their stimulatory effect on expiratory motor drive during cough and are a significant contributor to forming the cough motor pattern output (Pitts et al., 2016). Hanacek (1987) and Sant' Ambrogio et al. (1984) showed that blocking SARs, which are related to the Breuer-Hering reflex, with sulfur dioxide (SO₂), have a strong depressive influence on airway defense reflexes in rabbits (Hanacek, 1987; Sant' Ambrogio et al., 1984). However, SO₂ exposure can also activate C-fibers (Atzori et al., 1992; Canning et al., 2004; Wang et al., 1996) in guinea pigs and rats, and Tatar et al. (1988) demonstrated that activation of lung C-fibers can inhibit the cough reflex in the cat (Tatar et al., 1988). While it is clear that volume-related

feedback contributes to cough excitability in our model, the extent to which they directly contribute to evoking cough is unknown. While we did not test the effects of pulmonary stretch receptors on mechanically-induced laryngeal cough, Hanacek and colleagues have implicated pulmonary stretch receptors as having a role in cough excitability in chemically-induced laryngeal cough (Haná ek et al., 1984). These data suggest that volume-related feedback play a role in cough excitability regardless of cough type.

The mechanics of breathing with an open chest wall are very different from an intact chest wall. Following thoracotomy, we were able to generate cough, although excitability, specifically cough frequency, decreased. We observed a significant decrease in abdominal activity during these coughs, which yielded weaker, presumably less functional behaviors. It also is possible that abdominal pressure, which was not measured, decreased due to less effective compression of the chest cavity, thereby yielding a lower EMG magnitude. We cannot rule out the possibility that feedback from the chest wall may play a role in maintaining cough excitability. During the thoracotomy, incisions made separated the ribs, which could interrupt further chest wall dynamics. Large-amplitude chest wall vibration has been shown to decrease inspiratory motor output during breathing due to stimulation of intercostal muscle tendon organs (Bolser et al., 1988). Because cough CTI was largely unaffected, we believe that activation of costovertebral joint mechanoreceptors in the ribs are not an explanation for decreased cough excitability.

Our data have important implications for human lung transplant patients. Duarte and Myers (2012) concluded that the cough response is typically impaired for approximately 6–12 months in human lung transplant patients and that this is associated with impaired airway clearance (Duarte and Myers, 2012). Our data suggests that this impairment may be secondary to the functionally denervated status of the transplanted lung tissue, as there is no vagal afferent information coming from the transplanted lung tissue in a patient who has had a complete bilateral lung transplantation. We suggest that it is important for the lung transplant surgeons to preserve as much native airway tissue as medically and surgically feasible in order to optimize the cough response in these patients. Further investigation into the potential benefit of routine airway clearance in lung transplant patients may be beneficial.

4.1. Limitations

We cannot exclude the possibility of laryngeal stimulation affecting the tracheal or laryngeal mucosa. However, because our cannulas have a slight natural curve, only the smooth sides of the cannula come in contact with the mucosa. In our experience, the smooth part of the cannula against the tracheal mucosa does not elicit cough, and we do not believe the mucosa is damaged or irritated by this action.

Intrathoracic vagotomy was unable to be performed without first performing thoracotomy. Thoracotomy likely caused significant changes in the distribution of ventilation and perfusion in the lungs, although we hyperinflated the lungs and mitigated changes in blood gasses by adjustment of ventilator settings before the protocol began. Our approach did not address the potential contribution from altered chest wall mechanics on coughing. In animals for which trials with PEEP of 0 were conducted, cough excitability did not recover, even

after hyperinflation. However, we conducted the recovery cough trials within 10 min after hyperinflation. Because pulmonary stretch receptors recover slowly over a period of 20–30 minutes (Haná ek et al., 1984), the decrease in cough excitability may be a result of negative plasticity. We did not conduct LAR or CAP trials before thoracotomy due to the rapidly adapting nature of the cough reflexes.

Our experimental model was an anesthetized animal, and, therefore, we are limited in extrapolating our data to voluntary cough.

The vagus comprises a variety of fiber types, and a vagotomy is a non-insignificant injury. A reversible method such as an anesthetic nerve block, vagal cooling or optical inhibition might give additional insight as to which fiber types were responsible for cough permissibility and whether the damage induced by severing the vagus caused an artifactual decrease in cough excitability.

4.2. Conclusions

Our data suggest that vagal afferent information distal to the RLN, presumably volume-related feedback, has an excitatory or permissive effect on cough excitability irrespective of the source of the cough. The effect of vagotomy in this location is clinically relevant as this more closely simulates the point of functional vagotomy in human bilateral lung transplant recipients compared to prior studies. The cough response after bilateral lung transplantation is impaired with associated airway clearance impairment for at least 6–12 months in humans (Duarte and Myers, 2012).

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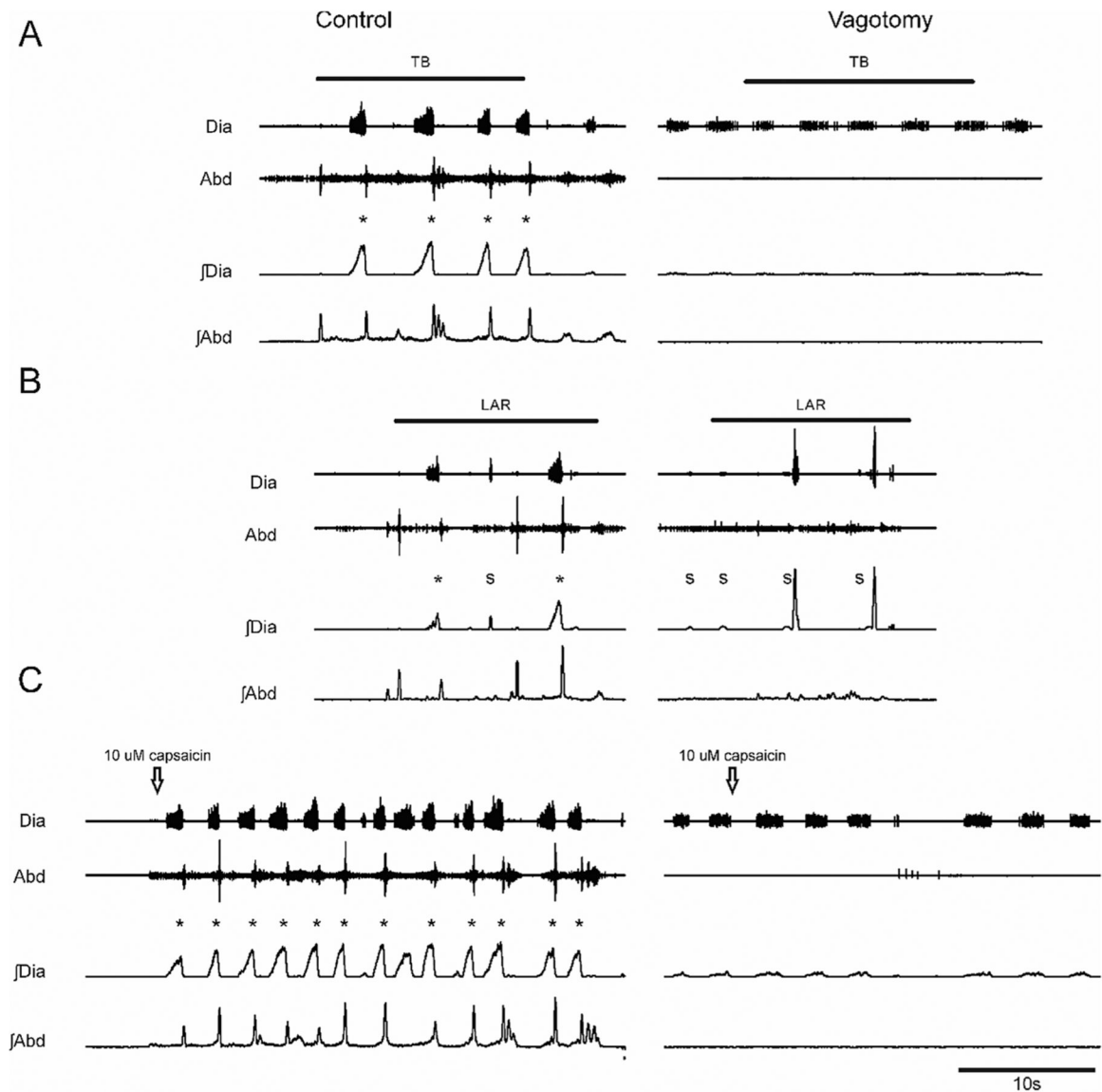


Fig. 1. Vagotomy decreases cough excitability.

Raw and integrated EMG data from diaphragm (Dia) and abdominal (Abd) muscle during (A) tracheobronchial (TB), (B) laryngeal (LAR) and (C) capsaicin-induced cough from control (post-thoracotomy/pre-vagotomy) and intrathoracic vagotomized conditions are shown from the same animal. EMG scales are the same between control and vagotomy conditions and between (A), (B) and (C). * indicates cough; ^s indicates swallow.

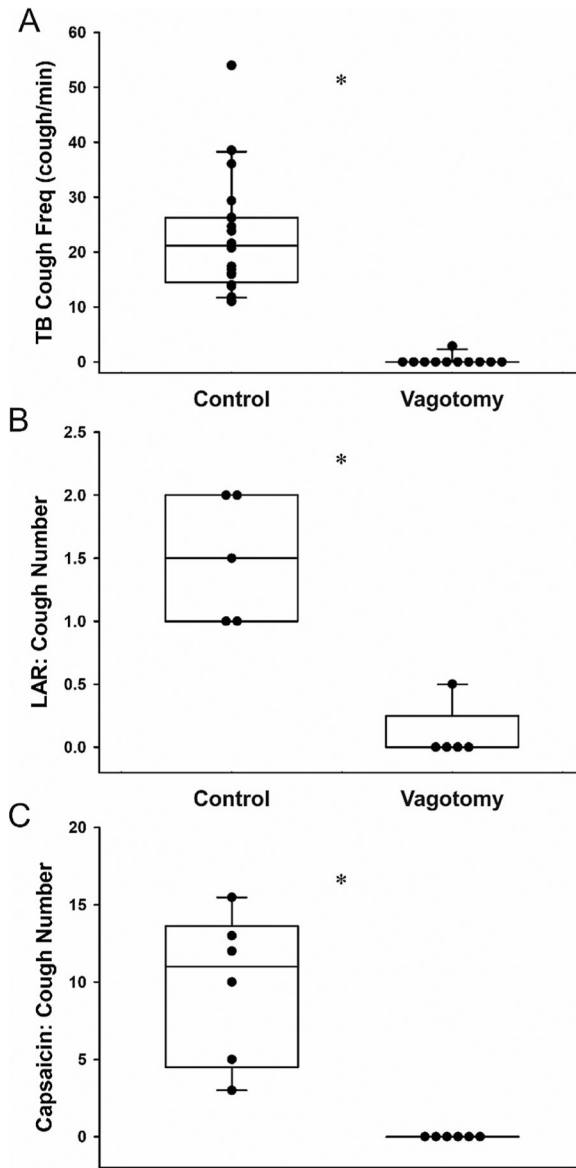


Fig. 2. Tracheobronchial (TB) and capsaicin-induced induced cough were abolished post-vagotomy. While not abolished, laryngeal-induced cough (LAR) was attenuated in the vagotomy condition. Changes in cough frequency of (A) TB cough and changes in cough number due to (B) laryngeal stimulation or (C) capsaicin due to vagotomy distal to the recurrent laryngeal nerve are compared to control (post-thoracotomy/pre-vagotomy). $\alpha = 0.05$, power = 1.0. TB – control: n = 20, vagotomy: n = 11, unpaired *t*-test, * $p < 0.001$; LAR control and vagotomy: n = 5, paired *t*-test, * $p = 0.002$; Capsaicin control and vagotomy: n = 6, paired *t*-test, * $p < 0.001$.

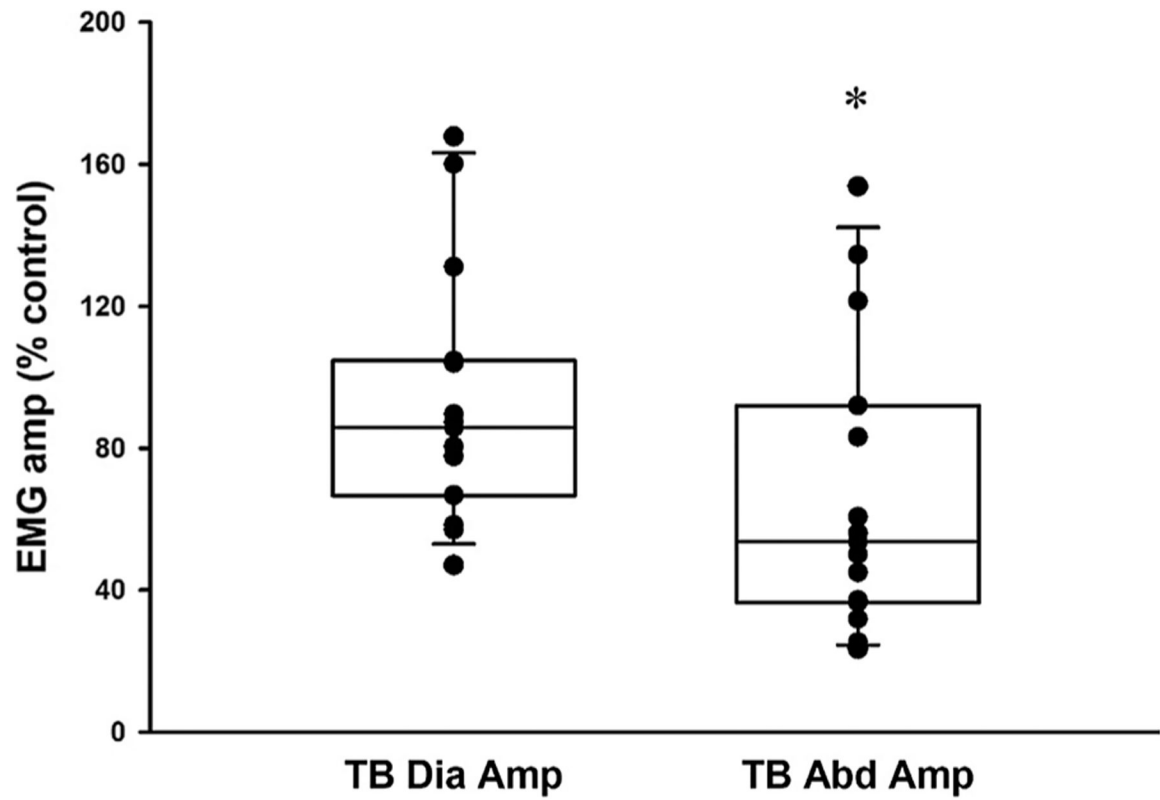


Fig. 3. Thoracotomy did not alter diaphragm magnitude during TB-induced cough, but abdominal muscle magnitude was significantly decreased relative to control.

* $p < 0.01$, $\alpha = 0.05$, power = 0.915, paired t -test against normalized control, $n = 15$.

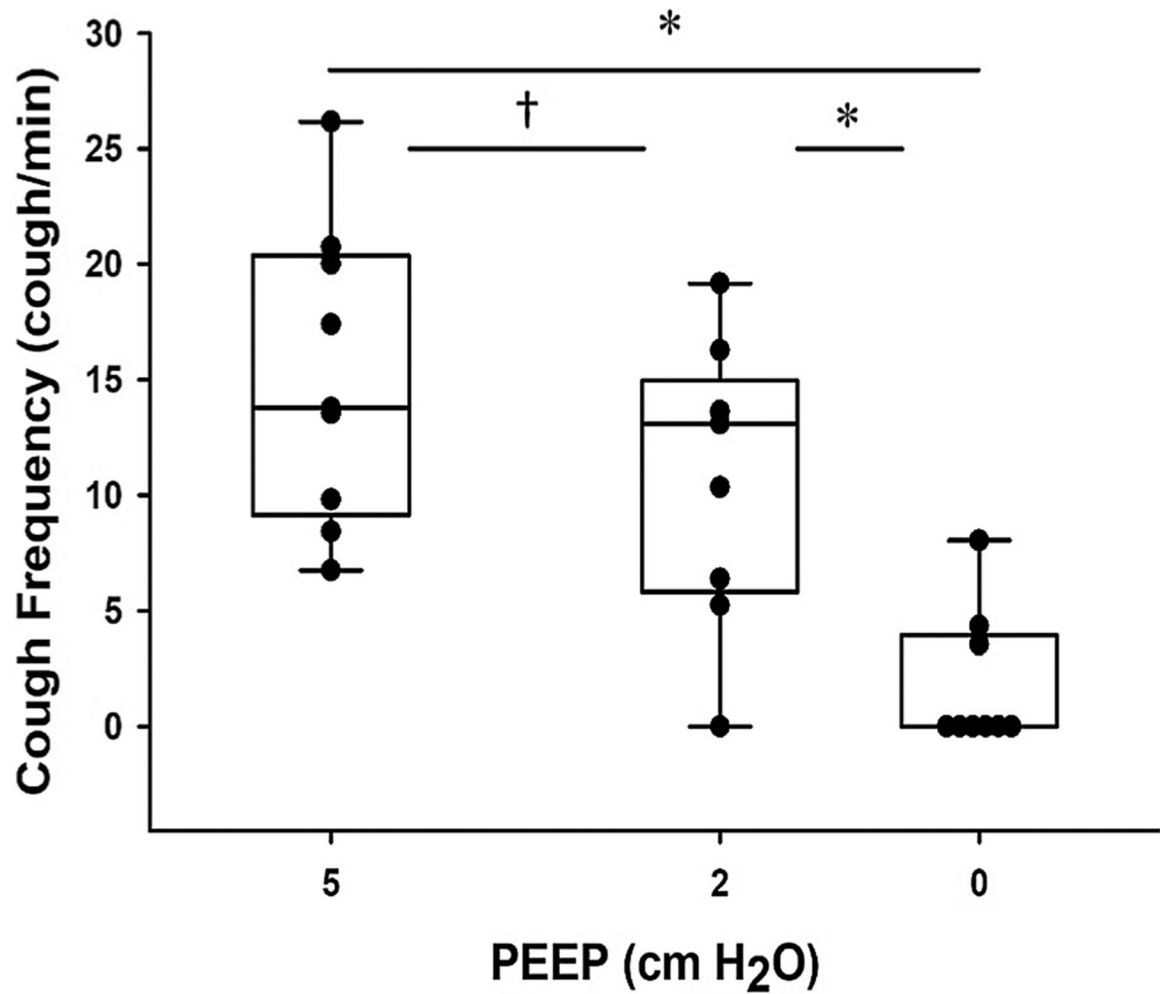


Fig. 4. Cough frequency decreased with decreasing positive end-expiratory pressure (PEEP). After thoracotomy, PEEP was used to maintain lung inflation. Cough frequency was significantly reduced at 0 cmH₂O PEEP relative to 5 and 2 cmH₂O (* $p < 0.05$, $\alpha = 0.05$, power = 0.999, repeated-measures ANOVA, Holm-Sidak post hoc, $n = 9$). There was a nonsignificant trend for cough frequency to be reduced between the 5 and 2 cmH₂O conditions († $p = 0.06$).