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Tutorial

Sensory Innervation of the Larynx and the Search for Mucosal Mechanoreceptors

Alexander G. Foote[a] and Susan L. Thibeault[a]

Purpose: The larynx is a uniquely situated organ, juxtaposed between the gastrointestinal and respiratory tracts, and endures considerable immunological challenges while providing reflexogenic responses via putative mucosal mechanoreceptor afferents. Laryngeal afferents mediate precise monitoring of sensory events by relay to the internal branch of the superior laryngeal nerve (iSLN). Exposure to a variety of stimuli (e.g., mechanical, chemical, thermal) at the mucosa–airway interface has likely evolved a diverse array of specialized sensory afferents for rapid laryngeal control. Accordingly, mucosal mechanoreceptors in demarcated laryngeal territories have been hypothesized as primary sources of sensory input. The purpose of this article is to provide a tutorial on current evidence for laryngeal afferent receptors in mucosa, the role of mechano-gated ion channels within airway epithelia and mechanisms for mechanoreceptors implicated in laryngeal health and disease.
Method: An overview was conducted on the distribution and identity of iSLN-mediated afferent receptors in the larynx, with specific focus on mechanoreceptors and their functional roles in airway mucosa.
Results/Conclusions: Laryngeal somatosensation at the cell and molecular level is still largely unexplored. This tutorial consolidates various animal and human researches, with translational emphasis provided for the importance of mucosal mechanoreceptors to normal and abnormal laryngeal function. Information presented in this tutorial has relevance to both clinical and research arenas. Improved understanding of iSLN innervation and corresponding mechanotransduction events will help shed light upon a variety of pathological reflex responses, including persistent cough, dysphonia, and laryngospasm.

Animals and humans have evolved diversity of specialized sensory cells to detect environmental physical stimuli, including heat, mechanical forces, chemical ligands, and light (Katta et al., 2015). Regulation of physiological processes in cells initiates at the periphery through mechanotransductive events, enacting signaling pathways for unique cell function and paracrine signaling to neural circuits—paramount for complex sensory systems. The larynx, similar to other well-established organs unifying the airway (e.g., lung), has been described as a highly responsive sensory organ. This emanates from its unique anatomic position and powerful reflexogenic mechanisms, which has received considerable attention for study of airway protective responses to stimulation of peripheral afferents in the internal superior laryngeal nerve (iSLN; Jafari et al., 2003; Sanders & Mu, 1998; Sant'Ambrogio & Widdicombe, 2001; Sinclair et al., 2017). The mucosa of the larynx contains one of the most dense concentrations of sensory receptors in the human body (Sanders & Mu, 1998), with a variety of physiological inputs from chemoreceptors (Jetté et al., 2020; Prescott et al., 2020), thermoreceptors (Sant'Ambrogio et al., 1985), proprioceptors (Bianconi & Molinari, 1962), nociceptors (Hamamoto et al., 2008, 2009; Uno et al., 2004; Yamamoto & Taniguchi, 2005), and mechanoreceptors (Andreatta et al., 2002; Davis & Nail, 1987; Hammer & Krueger, 2014). Laryngeal mechanoreceptors provide proprioceptive and perceptual afferent information for several essential human functions, including breathing, deglutition, speech, voice, and airway protection (Hammer & Krueger, 2014). Increased knowledge of mucosa mechanoreceptors in health and disease is paramount for finding novel treatment options for voice, swallow, and cough disorders. Insights into these elusive receptors require amalgamation of research from both animal and human models. Laryngeal mucosa is densely innervated with heterologous afferent receptor subtypes that exhibit specialized, albeit overlapping (i.e., polymodal), responses to physiological stimuli (Albegger et al., 1991; Domeij et al., 1991; Gonçalves da Silva Leite et al., 2016; Hisa et al., 1992; Jetté et al., 2020; Takahashi et al., 2016; Tanaka et al., 1993; Yamamoto et al., 2001, 1998, 2000, 1997). Data are often confounded with differences across species and uncertain

[a]Division of Otolaryngology–Head and Neck Surgery, University of Wisconsin–Madison

Correspondence to Susan L. Thibeault: thibeault@surgery.wisc.edu

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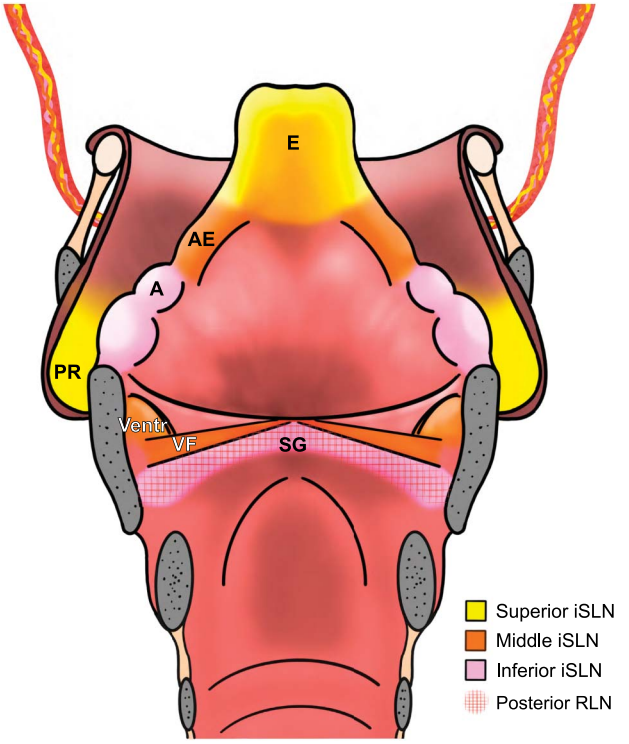
functional roles. Furthermore, we have little understanding of upstream transduction events at the laryngeal mucosa–airway interface for gating of mechanical stimuli. In this review, we evaluate our current knowledge of sensory innervation of the larynx with specific focus on mechanoreceptors for functional roles in health and disease. Our goal is to provide both clinicians and researchers in the field of voice with a comprehensive resource related to laryngeal sensory innervation in the context of normal and abnormal sensory pathophysiology. Details of mechano-gated ion channels for events of mechanotransduction at the mucosa–airway interface will be highlighted. Much research is warranted, and discoveries may provide targeted approaches for treating recalcitrant laryngeal pathologies.

Sensory Innervation of the Larynx

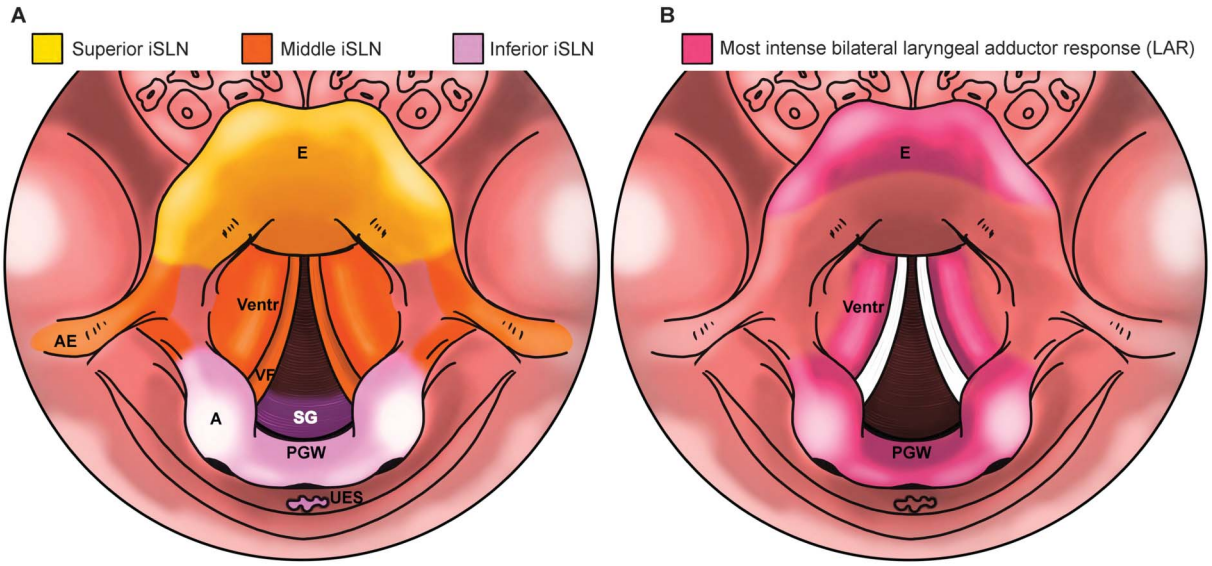
Anatomical and physiological knowledge of the SLN has been fundamental for our understanding of voice, swallow, and cough behaviors. The SLN and its functions, albeit physiologically important, remains poorly understood in the field of laryngology, which has hindered treatment options in the clinic. Despite much debate, recent work has indicated that the iSLN is sensory (Santoso et al., 2020), separate from the external branch, which supplies motor innervation to the cricothyroid muscle. iSLN innervation is distributed nonuniformly throughout the larynx (Boushey et al., 1974; Sanders & Mu, 1998; Sinclair et al., 2017), with stimulation of mucosa enacting a protective reflex—the laryngeal adductor response (LAR)—resulting in rapid vocal fold (VF) closure and tracheobronchial airway protection (Sinclair et al., 2017). These peripheral signals are conveyed to the central nervous system (CNS) via the iSLN branch of the vagus cranial nerve (X). Laryngeal mucosa afferents have first-order nerve cell bodies located in the nodose and jugular ganglion, which communicate via axon projections to second-order nerve cell bodies located within the interstitial subnucleus of the nucleus tractus solitarius (NTS) of the brainstem (Alvarez-Berdugo et al., 2016; Jürgens, 2002, 2009). In humans, the iSLN trunk enters through the thyrohyoid membrane and distributes into three major divisions (superior, middle, and inferior)—each demarcating distinct anatomic laryngeal territories, with individual branch subdivisions and ramified networks (see Figure 1; Sanders & Mu, 1998; Stephens et al., 1999). Despite minor anatomic discrepancies in the literature, the superior division supplies innervation to the mucosa of the laryngeal surface of the epiglottis and pyriform recess; the middle division supplies mucosa of the aryepiglottic folds, ventricular (false) folds, and true VFs; and the inferior division supplies the mucosa of the arytenoids, proximal subglottis, anterior wall of the hypopharynx (aka posterior glottic wall), and upper esophageal sphincter (see Figure 2A; Sanders et al., 1993; Stephens et al., 1999). While these formative studies provided important insights into laryngeal innervation, certain limitations merit discussion. The use of Sihler's staining technique allows for visualization of nerve distribution within soft tissues; however, the technique maps entire nerve supply patterns without

Figure 1. Animation of human larynx split open in posterior view. Internal superior laryngeal nerve (iSLN) enters the larynx through the thyrohyoid membrane and subdivides into three major divisions (superior, middle, and inferior), providing nerve supply to demarcated anatomic regions. E = epiglottis; AE = aryepiglottic fold; A = apex of arytenoid; Ventr = ventricular/false fold; VF = true vocal fold; PR = pyriform recess; SG = subglottic area; RLN = recurrent laryngeal nerve.



distinction between the SLN and the recurrent laryngeal nerve (RLN). The resulting data likely reflect an amalgam of both SLN and RLN nerve fibers. Only monosynaptic, antero- and/or retrograde tracing techniques (e.g., cholera toxin B subunit), which are prohibited for use in humans, are able to determine which afferent cell bodies innervate which receptors. Second, extensive individual variations in innervation patterns exist with the RLN and its branch subdivisions forming at least two anastomoses (i.e., interneural connections) at the level of the larynx (Naidu et al., 2014, 2012). The two most common interneural communications are Galen's anastomosis and the arytenoid plexus (Henry et al., 2017). Both exhibit high prevalence rates in humans and are believed to contribute to sensory innervation of the larynx via proprioceptive fibers that supply its joints and muscles (Henry et al., 2017; Sañudo et al., 1999). Galen's anastomosis forms by the union of the descending division of the iSLN and the posterior division of the RLN, after each extends off their muscular branches. Likewise, the arytenoid plexus forms from the union between the arytenoid branches of the iSLN and the anterior branch of the RLN. Although anastomoses have largely been studied by gross anatomical techniques in humans, these could produce

Figure 2. (A) Anatomic regions innervated via the human internal superior laryngeal nerve (iSLN). The iSLN subdivides into three major divisions (superior, middle, and inferior), which supply demarcated regions of the larynx with nonuniform heterologous nerve fibers. (B) Anatomic sites of most intense bilateral laryngeal adductor response (LAR) corroborate locations of highest nerve density. E = epiglottis; AE = aryepiglottic fold; A = arytenoid; VF = true vocal fold; Ventr = ventricular/false fold; PGW = posterior glottic wall; SG = subglottis; UES = upper esophageal sphincter.



considerable variability in the interpretation of both efferent and afferent innervation. For example, anterograde tracing techniques (i.e., cell body to nerve fiber projections) were utilized by injecting a label tracer into the nodose ganglion of the feline, which demonstrated that the posterior branch of the RLN provides additional sensory bilateral innervation to the caudal aspect of the VF and proximal subglottis via the ramus perforans (Yoshida et al., 2000, 1986). Additionally, complex anastomotic patterns exist, suggesting functional differences in sensory and motor innervation between individuals (Sañudo et al., 1999). The importance of sensory afference cannot be understated as surgical bilateral denervation of the iSLN abolishes the LAR with significant loss of neural innervation to supraglottic and glottic regions and partial loss to the subglottis (Matsuo & Shin, 1994; Sasaki et al., 2005).

Physiological Data Suggest Evidence for Mechanoreceptors in Laryngeal Mucosa

To date, researchers have utilized anatomical, behavioral, and neurophysiological methods to elucidate defined areas exhibiting differential densities of neural innervation and sensory response to mechanical perturbation (Sanders & Mu, 1998; Sinclair et al., 2017; Yamashita et al., 1997; Yoshida et al., 1986). The role of sensory receptors involved remains elusive. Accordingly, rapidly adapting mucosal mechanoreceptors have been implicated in elicitation of the LAR (Andreatta et al., 2002). This involuntary airway protective reflex is highly conserved among species (Andreatta et al., 2002; Sinclair et al., 2017). The LAR consists of a rapid ipsilateral thyroarytenoid (TA) response, R1 (approximately 15–18 ms after stimulus), and a delayed, independent bilateral TA response, R2 (approximately 60–70 ms; Sinclair et al., 2017; Yamashita et al., 1997). While the rapid R1 response has been largely attributed to mucosal mechanoreceptor afferents, research suggests more centralized regulation of the delayed R2 response (Barkmeier et al., 2000; Sasaki et al., 2003). Other work has demonstrated clear physiological differences in the characteristics of electromyography response to electrical stimulation of the iSLN from that of responses to air puff stimulation of laryngeal mucosa (Bhabu et al., 2003; Kearney et al., 2005). Activation of mechanoreceptors by direct air puff stimulation to mucosa in humans only elicits a bilateral, late response around 100 ms or greater, whereas electrical stimulation of the iSLN produces an early ipsilateral response in the TA around 16 ms and bilateral R2 responses around 65 ms (Ludlow et al., 1992). Given that the early R1 response is only elicited upon electrical stimulation, it has been argued that this is an artificial response due to direct electrical nerve stimulation, and only the delayed, bilateral R2 response is similar to what occurs with mechanoreceptor stimulation as both occur between 60 and 100 ms poststimuli. This distinction is remarkable, as electrical stimulation of a nerve can induce a response that normally does not occur in nature. Similar differences have been exhibited between the blink reflex elicited by electrical or mechanical stimulation of the supraorbital nerve and responses to air puff stimuli to the cornea in humans (Esteban, 1999). Studies investigating the effect of gating of the LAR during voicing and breathing tasks further attest to this distinction. When electrical stimulation

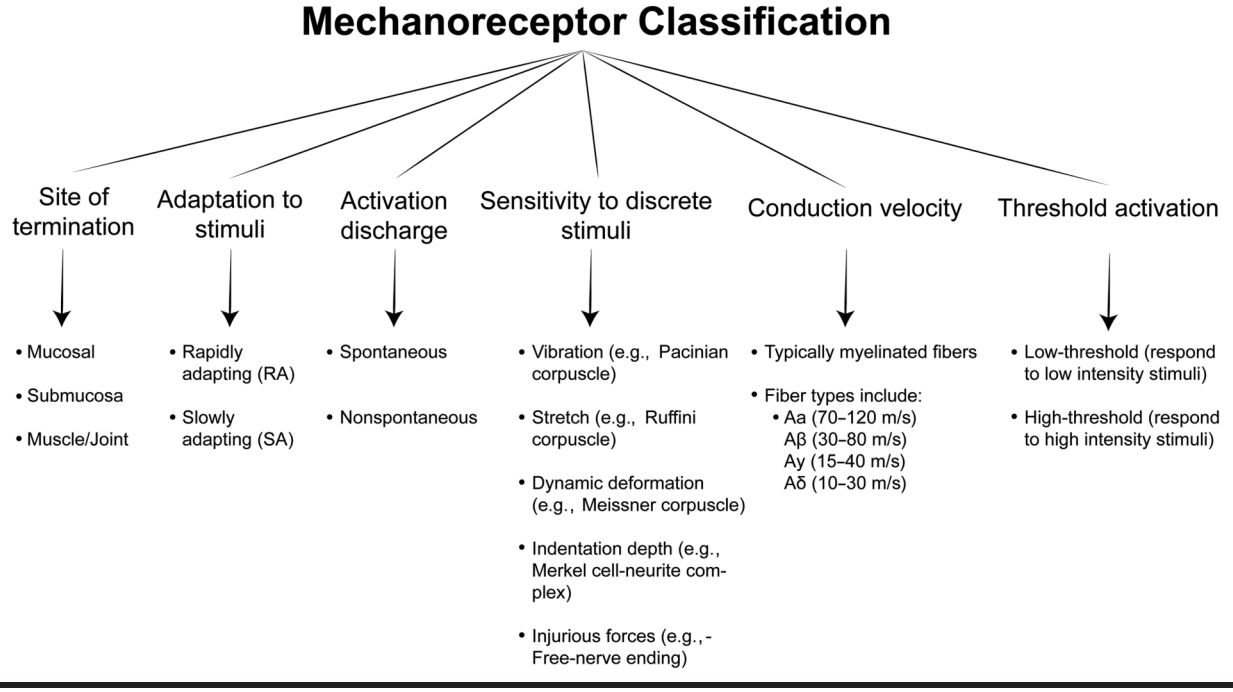
of the iSLN was tested during various activities (e.g., voicing, inspiration/expiration, and breath hold), no effects were found on R1 responses, implying that electrical elicitation was unmodified by central activation (Henriquez et al., 2007). On the contrary, modulation of mechanosensory afference was altered via air puff pressure stimulation to arytenoid mucosa, demonstrating increased thresholds for eliciting LAR during voicing (Hammer & Krueger, 2014). Interpretation of the R1 response elicited by electrical stimulation or mechanical stimulation to nerve endings (Andreatta et al., 2002) should be approached with caution. This may produce adductor responses that are unlikely to be functionally relevant to laryngeal protective reflexes in awake humans and may be the result of electrical stimulation likely activating mechanoreceptor afferents along with C-fibers and capsaicin-sensitive receptors, while the air pressure puff technique is likely to only activate mechanoreceptors.

Mechanoreceptors have historically been categorized based upon sites of termination, rapidly or slowly adapting, spontaneous activity, sensitivity to discrete mechanical stimuli, conduction velocity (i.e., myelinated, A-range), and low- versus high-threshold activation response (see Figure 3; Boushey et al., 1974; Bradley, 2000; Davis & Nail, 1987; Sant'Ambrogio & Widdicombe, 2001; Tsuda et al., 1998). Mechanoreceptors in the larynx have been differentiated by location, either in the superficial mucosa (i.e., mucosal mechanoreceptors) or deeper in muscles and laryngeal joints (i.e., proprioceptive afferents; Bradley, 2000). To this end, elicitation of LAR responses, in the feline, was found to be highly dependent on the presence of mucosal mechanoreceptors and not proprioceptive afferents following perturbation pre/post mucosal strip conditions (Andreatta et al., 2002). The classical hallmark of mechanoreceptors is that they exhibit rapid adaptation upon stimulation (Sant'Ambrogio & Widdicombe, 2001), with previous research indicating a significant proportion of laryngeal mechanoreceptor responses were rapidly adapting when recorded from the SLN (Davis & Nail, 1987) or presynaptically in the NTS of the brainstem (Esaki et al., 1997).

Much work has been done to establish territorial differences regarding density of neural innervation and analogous sensory responses. In excised human larynges, a rich distribution of nerve endings to mucosa of the ventricular and true VF, arytenoid, posterior glottis, and laryngeal surface of the epiglottis were found (see Figure 2A; Sanders & Mu, 1998). While authors assumed data to reflect density of mechanoreceptors, distinction between chemo- and mechanoreceptors was not considered and currently remains unclear, as many receptors function as polymodal—responding to a wide variety of stimuli, including mechanical, chemical, and thermal (Sant'Ambrogio & Widdicombe, 2001). Using anterograde tracing techniques in the feline, injection of wheat germ agglutinin–horseradish peroxidase into the nodose ganglion demonstrated distribution of laryngeal peripheral sensory nerve fibers to the epiglottis, aryepiglottic fold, arytenoid eminence, rostral 1/3 VF, and laryngeal vestibulum. Areas of greatest sensory supply were observed on laryngeal aspect of the epiglottis, aryepiglottic fold, and arytenoid region, reflecting anatomical locations involved in reflex closure of the glottis (Yoshida et al., 1986).

Anatomic studies largely correspond to physiological data demonstrating LAR-elicited sensory topography of

Figure 3. Classification of mechanoreceptor phenotypes.



the human larynx (see Figure 2B; Sanders & Mu, 1998; Sinclair et al., 2017). Using electrical stimulation of the laryngeal mucosa, the researchers mapped LAR elicitation in various subsites, including epiglottic tip, membranous true VF, midventricular VF, and posterosuperior supraglottis. It was found that elicitation of bilateral LAR responses via electrical mucosal stimulation was predominate in the laryngeal subsites of posterosuperior supraglottis followed by epiglottic tip and ventricular VF, albeit absent in membranous true VF, subglottis, and epiglottic petiole (Sinclair et al., 2017). Authors speculated that differential reflex responses likely evolved to protect against inappropriate LAR activation during volitional tasks without compromise of airway protection. Of particular interest was the lack of LAR response in the membranous VF subsite (Sinclair et al., 2017), despite the previous findings of neural endings in this region (Sanders & Mu, 1998). Given the potential for biomechanical trauma induced to the membranous VF during phonation and cough, this may suggest that identity and function of intra- and subepithelial nerve fibers likely serve as high-threshold, mono- or polymodal nociceptors. Considered together, data might suggest an evolved physiological advantage for demarcated density and distribution of afferent fibers in humans.

Species and Regional Differences in Laryngeal Afferent Nerve Fibers

Extensive histological studies have been performed in a wide variety of animal models to explore identity and function of sensory afferent receptors in laryngeal mucosa (see Table 1; Albegger et al., 1991; Domeij et al., 1991; Gonçalves da Silva Leite et al., 2016; Hisa et al., 1992; Jetté et al., 2020; Takahashi et al., 2016; Tanaka et al., 1993; Yamamoto et al., 2001, 1998, 2000, 1997). However, morphological, topographical, and physiological characteristics of these structures have not been definitively categorized. Species differences are commonplace; therefore, it is important to understand and consider the limitations of specific animal models and different species offer differing advantages, depending on the end points of the investigation. Historically, the feline (cat) model has been utilized for the study of laryngeal mechanoreception (Andreatta et al., 2002; Davis & Nail, 1987; Sampson & Eyzaguirre, 1964). This is due to the high number of myelinated fibers in the feline vagus nerve, averaging 30,000 afferent nerve fibers in the vagus trunk, with 3,000 myelinated fibers in the SLN branch, of which 2,400 are afferent (Jammes et al., 1982; Sant'Ambrogio & Widdicombe, 2001; Tsuda et al., 1998). A preponderance of myelinated fibers occurs in the SLN of humans, felines, and canines (Sant'Ambrogio & Widdicombe, 2001), with similar focal areas of rich innervation exhibited for human and feline larynges (Andreatta et al., 2002; Yoshida et al., 1992). Furthermore, in the feline, monomodal mechanosensitive fibers were predominately demonstrated in afferents of the SLN compared to chemosensitive and polymodal fibers (Takagi et al., 1995). This model has proved effective to study mucosal mechanoreceptor phenotypes and for accurate modeling of sensitivity to mechanical perturbations contributing to movement dynamics. In addition to feline (Tanaka et al., 1993), canine (Yamamoto et al., 1997), equine (Yamamoto et al., 2001), and rodent (e.g., mouse, rat) models have been predominately utilized for morphological studies of nerve endings in laryngeal mucosa (Lima-Rodrigues et al., 2004; Sbarbati et al., 2004; Soda & Yamamoto, 2012; Takahashi et al., 2016; Yamamoto et al., 1998, 2000, 2003). In light of the vast array of transgenic tools available today, the mouse can be a very powerful model for study of mechanosensory protein and neuron activation (Kichko et al., 2015), with numerous similarities to humans, albeit few studies have explored receptor afferents using this model (Hamamoto et al., 2008; Jetté et al., 2020; Prescott et al., 2020). In comparison, neurophysiology of the rat has been well characterized with ample insight into laryngeal sensory receptor afferents but currently lacks accessible transgenic models. Due to advancements in genomics (i.e., CRISPR/Cas9), alongside technological innovation (i.e., single-cell transcriptomics, optogenetics, antero- or retrograde cell tracing), research trends appear to emphasize increased use of transgenic mouse models based on both precedent literature and ethical concerns (Birling et al., 2017; Ellenbroek & Youn, 2016; Kim et al., 2016; Mahmoudi et al., 2017). Aforementioned techniques allow for targeted manipulation of specific cell types and neural pathways in readily available genetically engineered mice to elucidate biological mechanisms. Use of these techniques during developmental stages may help to define the molecular profiles for placode versus neural crest cell contributions to the intrinsic and extrinsic laryngeal nervous system. Insights will aid in our understanding of iSLN and RLN physiology and may provide answers for commonly observed pediatric disorders, such as congenital laryngomalacia and idiopathic VF paralysis, given their high prevalence and assumed neurological etiology (Hsu et al., 2015; Thompson, 2007). For example, recent data have identified different mutations found responsible for what was previously thought to be idiopathic VF paralysis. In one study, a mutation in the coding for dynactin, an axonal transport protein, was found affected in families with VF paralysis (Puls et al., 2003, 2005), while in other studies, a mutation affecting transient receptor potential vanilloid 4 (TRPV4) was found responsible for familial VF paralysis in Charcot-Marie-Tooth Type 2C (Landouré et al., 2012, 2010; Zimon et al., 2010).

Many sensory receptor subtypes exist in laryngeal mucosa, with elicitation of several different reflexes (Widdicombe, 1998). Prior work has indicated free nerve endings of myelinated or unmyelinated nerve fibers reside in laryngeal epithelium and respond to mechanical and/or chemical stimuli, generally regarded as polymodal nociceptors (Boushey et al., 1974; Davis & Nail, 1987; Tsuda et al., 1998). It is generally accepted that laryngeal chemoreflexes originate from free nerve endings of unmyelinated fibers (type C-fibers; Sbarbati et al., 2004; Yamamoto et al., 2000), while mechanoreflexes usually involve thin myelinated fibers (type Aδ or Aβ) with or without complex nerve endings (i.e., corpuscular, laminar, glomerular, lamellar) and with rapid adaptation

Table 1. Species and regional differences in laryngeal afferent nerve fibers.

					Laryngeal mucosa							
				Location:	mVF	cVF	Laryngeal epiglottic surface	Ventr fold	Ary-epiglottic fold	Arytenoid	Posterior glottic wall	Proximal subglottic
Neuropeptide or molecule	**Reported receptor class**	**Labels**	**Species**	***Epithelia:***	**Stratified squamous**		**Stratified squamous, intermediate, ciliated columnar**	**Ciliated pseudostratified**		**Nonciliated cuboidal**	**Ciliated pseudostratified**		**Cited literature**
SP	C-fiber peptidergic nociceptor	Neurons, sub- and intraepithelial free-ended nerve fibers, and taste bud–like structures	human (H), rat (R), mouse (M), feline (F), dog (D), equine (E)		R	R, F, D	H, R, F, D, E	H, F, D	R, F, E	R, M, F, D, E	R, F, E	R, F, D, E	Albegger et al., 1991; Corcoran et al., 1999; Domeij et al., 1991; Hamamoto et al., 2009; Kawasoe et al., 1990; Lima-Rodrigues et al., 2004; Nishijima & Atoji, 2004; Shin et al., 1987; Takahashi et al., 2016; Tanaka et al., 1993; Yamamoto et al., 2001, 2003; Yoshida et al., 2000
CGRP	C-fiber peptidergic nociceptor	Neurons, sub- and intraepithelial free-ended nerve fibers, and chemosensory cells	human (H), rat (R), feline (F), dog (D), equine (E)		H, R, F, D	H, R, F, D	R, F, D, E	H, F, D	R, F, E	R, D, E	R, F, D, E	R, F, D, E	Albegger et al., 1991; Corcoran et al., 1999; Domeij et al., 1991; Hisa et al., 1992; Kawasoe et al., 1990; Lima-Rodrigues et al., 2004; Matsuo & Shin, 1994; Nishijima & Atoji, 2004; Takahashi et al., 2016; Tanaka et al., 1993; Yamamoto et al., 2001, 2003; Yoshida et al., 2000

(*table continues*)

Table 1. *(Continued).*

				Laryngeal mucosa								
				Location:	**mVF**	**cVF**	**Laryngeal epiglottic surface**	**Ventr fold**	**Ary-epiglottic fold**	**Arytenoid**	**Posterior glottic wall**	**Proximal subglottic**	
Neuropeptide or molecule	**Reported receptor class**	**Labels**	**Species**	***Epithelia:***	**Stratified squamous**		**Stratified squamous, intermediate, ciliated columnar**	**Ciliated pseudostratified**		**Nonciliated cuboidal**	**Ciliated pseudostratified**		**Cited literature**
VIP	Class II G-protein–coupled receptor	Apical visceral mucosa, intestinal epithelial cells	dog (D), equine (E)							E		D	Corcoran et al., 1999; Kawasoe et al., 1990
Neurokinin A	Nociceptor	Neurons and secretory cells	human (H)		H	H		H					Albegger et al., 1991
PGP9.5	Polymodal nociceptor	Neurons and intraepithelial free-ended nerve fibers	human (H), rat (R) mouse (M), feline (F), equine (E)		H, F	H, F	H, R, E	H	R, E	H, R,M, E	F	F	Gonçalves da Silva Leite et al., 2016; Jetté et al., 2020; Matsuo & Shin, 1994; Nishijima & Atoji, 2004; Ruoppolo et al., 2015; Soda & Yamamoto, 2012; Yamamoto et al., 2001, 2003
NFP	n/a	Type IV intermediate filaments in neurons and nerve fibers	dog (D), equine (E)				D, E			D	D		Yamamoto et al., 2001, 1997
P2X3	Ionotropic purinergic receptor	Neurons and intraepithelial ramified nerve fibers or associated with chemosensory cells and neuroendocrine cells	human (H), rat (R), mouse (M)				R			H, R, M	R	R	Jetté et al., 2020; Soda & Yamamoto, 2012; Takahashi et al., 2016
P2X2	Ionotropic purinergic receptor	Sensory neurons and nerve fibers	rat (R)							R		R	Takahashi et al., 2016

(*table continues*)

Table 1. *(Continued).*

					Laryngeal mucosa							
				Location:	**mVF**	**cVF**	**Laryngeal epiglottic surface**	**Ventr fold**	**Ary-epiglottic fold**	**Arytenoid**	**Posterior glottic wall**	**Proximal subglottic**	
Neuropeptide or molecule	**Reported receptor class**	**Labels**	**Species**	***Epithelia:***	**Stratified squamous**		**Stratified squamous, intermediate, ciliated columnar**	**Ciliated pseudostratified**		**Nonciliated cuboidal**	**Ciliated pseudostratified**		**Cited literature**
P2RY1	G-coupled purinergic receptor	Second-order chemosensory neurons that form corpuscle terminals that appose laryngeal taste buds	mouse (M)		M	M	M		M	M		M	Prescott et al., 2020
SNAP25	Presynaptic plasma membrane protein	Neurons and nerve fibers, subpopulation of Type III cells and neuroendocrine cells	human (H), rat (R), mouse (M)				R			H, R, M		R	Jetté et al., 2020; Takahashi et al., 2016
Calbindin 1 (CALB1)	*Unclear*	Intracellular calcium in neurons and laminar nerve endings associated with taste buds, sub- and intraepithelial nerve fibers, and endocrine cells	rat (R), mouse (M)				R, M			R		M	Nishijima & Atoji, 2004; Prescott et al., 2020; Yamamoto et al., 2000
Calretinin	*Unclear*	Intracellular calcium in neurons and sub- and intraepithelial nerve fibers with laminar endings	rat (R)				R		R	R			Soda & Yamamoto, 2012; Yamamoto et al., 1998
vGLUT1	Transporter	Neurons and endocrine cells	rat (R)				R			R	R		Soda & Yamamoto, 2012; Takahashi et al., 2016
vGLUT2	Transporter	Neurons and endocrine cells	rat (R)				R			R	R		Soda & Yamamoto, 2012; Takahashi et al., 2016

(*table continues*)

Table 1. *(Continued).*

					Laryngeal mucosa							
				Location:	mVF	cVF	Laryngeal epiglottic surface	Ventr fold	Ary-epiglottic fold	Arytenoid	Posterior glottic wall	Proximal subglottic
Neuropeptide or molecule	**Reported receptor class**	**Labels**	**Species**	***Epithelia:***	**Stratified squamous**		**Stratified squamous, intermediate, ciliated columnar**	**Ciliated pseudostratified**		**Nonciliated cuboidal**	**Ciliated pseudostratified**		**Cited literature**
vGLUT3	Transporter	Neurons and endocrine cells	rat (R)							R	R		Takahashi et al., 2016
Na^+/K^+ ATPase, α3-subunit	Mechanoreceptor	Neurons and subepithelial nerve fibers with laminar endings	rat (R)				R						Soda & Yamamoto, 2012
S-100	n/a	Schwann cells	rat (R)				R						Soda & Yamamoto, 2012
Myelin basic protein		Myelinated axons	rat (R)							R			Takahashi et al., 2016
GNAT3	Chemoreceptor	G-protein subunit in Type II taste cells and solitary chemosensory cells	human (H), rat (R), mouse (M)				R		R	H, R, M	R	R	Jetté et al., 2020; Sbarbati et al., 2004; Takahashi et al., 2016
PLCβ2	Chemoreceptor	Transduction component in Type II taste cells	human (H), rat (R), mouse (M)				R		R	H, R, M			Jetté et al., 2020; Sbarbati et al., 2004
CA4	Chemoreceptor	Sour-responsive Type III taste cells	human (H)							H			Jetté et al., 2020
Acetylated tubulin		Nerve fibers	human (H), mouse (M)							H, M			Jetté et al., 2020
NPY1R	G-protein–coupled receptor	Jugular-derived neurons with free-ended nerve fibers	mouse (M)				M			M			Prescott et al., 2020
NPY2R	G-protein–coupled receptor	Nodose-derived neurons with free-ended nerve fibers	mouse (M)									M	Prescott et al., 2020
TRPV1	Polymodal nociceptors	Ca^{2+}-gated mechanosensitive transmembrane ion channels in	human (H), rat (R), mouse (M)		M	M	H, R, M			R, M		R, M	Hamamoto et al., 2008, 2009; Uno et al., 2004; Yamamoto & Taniguchi, 2005

(*table continues*)

Table 1. *(Continued).*

					Laryngeal mucosa							
				Location:	mVF	cVF	Laryngeal epiglottic surface	Ventr fold	Ary-epiglottic fold	Arytenoid	Posterior glottic wall	Proximal subglottic
Neuropeptide or molecule	**Reported receptor class**	**Labels**	**Species**	***Epithelia:***	**Stratified squamous**		**Stratified squamous, intermediate, ciliated columnar**	**Ciliated pseudostratified**		**Nonciliated cuboidal**	**Ciliated pseudostratified**		**Cited literature**
TRPV2		neurons and various cell types	human (H), rat (R), mouse (M)		M	M	H, R, M			R, M		R, M	Hamamoto et al., 2008, 2009; Uno et al., 2004; Yamamoto & Taniguchi, 2005
TRPV3			human (H), mouse (M)		M	M	H, M			M		M	Hamamoto et al., 2008, 2009
TRPV4			human (H), mouse (M)				H						Hamamoto et al., 2008, 2009
αENaC	Mechano- and chemoreceptors	Na^+-gated transmembrane ion channels in neurons, epithelia, and taste buds	rat (R)				R						Yamamoto & Taniguchi, 2006
βENaC			rat (R)				R						Yamamoto & Taniguchi, 2006
γENaC			rat (R)				R						Yamamoto & Taniguchi, 2006

Note. mVF = membranous vocal fold; cVF = cartilaginous vocal fold; Ventr fold = ventricular fold; SP = substance P; CGRP = calcitonin gene-related peptide; VIP = vasoactive intestinal polypeptide; PGP9.5 = protein gene product; NFP = neurofilament protein; P2X3 = purinergic receptor P2X, ligand-gated ion channel, 3; P2X2 = purinergic receptor P2X, ligand-gated ion channel, 2; P2RY1 = purinergic receptor P2Y1; SNAP25 = synaptosomal-associated protein, 25 kDa; vGLUT = vesicular glutamate transporter; GNAT3 = α-gustducin; PLCβ2 = phospholipase C β2; CA4 = carbonic anhydrase 4; NPY1R = neuropeptide Y receptor Type 1; NPY2R = neuropeptide Y receptor Type 2; TRPV = transient receptor potential vanilloid; ENaC = epithelial sodium channel.

(Sant'Ambrogio & Widdicombe, 2001; Yamamoto et al., 1997). The most investigated neuropeptide across animal models was found to be the free-ended C-fiber–associated nociceptors, substance P (SP), and calcitonin gene-related peptide (CGRP)—localized to sub- and intraepithelial structures (Corcoran et al., 1999; Domeij et al., 1991; Hayakawa et al., 2014; Hisa et al., 1992; Kawasoe et al., 1990; Shin et al., 1987; Tanaka et al., 1993). Generally, across studies, density of nerve fibers was found to increase in the supraglottis than in the subglottis and in the posterior compared to the anterior glottic regions. In specific, territorial differences were observed with increased fiber density in the posterior 1/3 cartilaginous VF, anterior wall of the hypopharynx (posterior glottic wall), and arytenoid mucosa. While the anterior 2/3 membranous VF exhibited innervation, fiber density was marginal. The highest density of pan-neuronal marker protein gene product (PGP9.5) was observed just anterior to the vocal process in the feline VF (Matsuo & Shin, 1994), which suggests an important perceptive site within the anterior glottis. Anatomic distribution of neuropeptides and molecules across animal models was quite similar, with the rat being the most investigated model across studies. While human studies were scarce, recent work has demonstrated intraepithelial free nerve endings and glomerular endings via PGP9.5 reactivity in VF mucosa and PGP9.5 and P2X3 in arytenoid mucosa of human newborns, as well as TRPV1–TRPV4 protein isoforms in the human adult epiglottis (Gonçalves da Silva Leite et al., 2016; Hamamoto et al., 2009; Jetté et al., 2020).

These collective immunohistochemical studies corroborate human physiological data establishing territorial demarcations for afferent nerve fiber densities and LAR sensory receptors. Data support the evolved importance of focal sensory mechanisms in the larynx that serve as checkpoints to inhibit unnecessary reflexogenic responses during volitional and nonvolitional tasks while also allowing for adequate upper airway protection. Human studies are rare; thus, there are not enough data to support a clear correlation to animal models. However, there seems to be agreement on the importance of somatosensory perception to the superior/posterior glottis, with assumptions given to the role in eliciting rapid reflexive responses for airway protection, while also attenuating sensory perception in focal regions of high mechanical activity within the anterior glottis.

Mechano-Gated Ion Channels Provide Key Insight for Transduction Events

Cells actively sense and respond to their physical environment through events of mechanotransduction, providing the molecular machinery to cells for sensory detection and subsequent information relay from peripheral nerve endings. In biology, this involves translating mechanical stresses into biochemical signals, thus enabling cells to adapt to their surrounding tissue environment (Jaalouk & Lammerding, 2009). While numerous studies have provided biological evidence for nerve terminals in laryngeal mucosa, we have little understanding of upstream transduction events at the mucosa–airway interface. The ability of mechanoreceptors to detect mechanical cues relies on the presence of mechanotransducer ion channels to rapidly transform mechanical forces into electrical signals and depolarize local receptive fields. This local depolarization can generate action potentials (i.e., receptor potential) that propagate toward the CNS (Roudaut et al., 2012). However, properties of molecules that mediate mechanotransduction and adaptation to mechanical forces remain vaguely defined in the larynx. The emergence of novel ion channels as candidates for transduction molecules has provided critical insight into somatosensory processing for sensory organs.

To date, proprioceptors and mechanoreceptors in human upper airway have been localized to pharyngeal walls (Pacini-like corpuscles, Ruffini-like corpuscles, spiral-wharves nerve structures; de Carlos et al., 2013), VF mucosa (intraepithelial free nerve endings, glomerular endings), and laryngeal intrinsic muscles (Meissner-like corpuscles, muscle spindles, spiral-wharves nerve structures; Gonçalves da Silva Leite et al., 2016). Mechano-gated ion channels (i.e., mechanoproteins) have been found in neurons within vagal sensory ganglia (nodose/jugular/petrosal superganglia; Lu et al., 2009; Nonomura et al., 2017; Prescott et al., 2020; Zeng et al., 2018; L. Zhang et al., 2004) and at mucosal surfaces of the unified airway—particularly in epithelia (de Carlos et al., 2013; Groneberg et al., 2004; Hamamoto et al., 2008, 2009; Stewart & Davis, 2019). Airway epithelia are mechanically sensitive cell types, which, in addition to their protective roles, perform key signaling functions for regulation of mucosal health (Althaus et al., 2007; Button et al., 2013; Eisenhoffer et al., 2012; van der Vliet & Bove, 2011). Identification of mechanoproteins with putative importance for laryngeal function includes the family of transient receptor potential channels (e.g., TRPV1–TRPV4, transient receptor potential ankyrin 1 [TRPA1]; Groneberg et al., 2004; Hamamoto et al., 2008, 2009; L. Zhang et al., 2004), the degenerin/epithelial sodium channels (Leydon et al., 2009; Tsujimura et al., 2019), and its subfamily acid-sensing ion channels (ASICs, e.g., ASIC2; de Carlos et al., 2013; Lu et al., 2009). Epithelial sodium channels (ENaCs) contribute to a variety of sensory perceptions such as touch, smell, vision, hearing, salty taste, and sensation of temperature changes (Qadri et al., 2012). ENaCs are expressed in rat sensory neurons within nodose ganglion and in nerve endings of the laryngeal submucosal layer and epiglottic mucosa (Yamamoto & Taniguchi, 2006). Mechanical activation of ENaCs is essential for electrolyte and water balance and has been found, alongside sodium–potassium pumps (Na^+/K^+ ATPase), to regulate cell volume by supporting active transport across cells (Althaus et al., 2007; Fisher et al., 2001; Leydon et al., 2009). The ENaC has also been involved in initiation of mechanically evoked (i.e., punctate, pressure) swallows in anesthetized rats, albeit highly dependent on SLN integrity (Tsujimura et al., 2019). These researchers further demonstrated topical application to laryngeal mucosa of either an ENaC blocker (amiloride) and its analogs (benzamil and dimethyl amiloride) and/or a mechanosensitive channel blocker (gadolinium) inhibited mechanically elicited swallows, but not by ASIC drug antagonists

(mambalgine-1 and diminazene; Tsujimura et al., 2019). However, ASICs and TRPV1 exhibit activation following acid exposure with acid-evoked swallows abrogated by the combination of both drug antagonists, suggesting their possible role in gastroesophageal reflux disease (GERD; Tsujimura et al., 2019). Purinergic signaling (P2Y subclass) via adenosine triphosphate release has also been found to promote repair in human airway epithelia and maintain proper mucus hydration of lower airways (Button et al., 2013; van der Vliet & Bove, 2011). Recent work in mice has demonstrated the critical role P2RY1 sensory neurons play in airway defense, functioning as second-order chemosensors in the larynx (Prescott et al., 2020). Researchers utilized sweeping genetic approaches to identify diversity of neuron populations within nodose/jugular/petrosal superganglia and analogous laryngeal terminals via Cre-dependent adeno-associated viruses encoding reporter biomarkers. Results found that physiological responses to laryngeal acid and water challenge required P2RY1 neurons and evoked a coordinated motor program for airway defense, including not only apnea but also VF adduction, pharyngeal swallow, and expiratory reflexes. Purinergic receptors (P2X subclass) have also been previously localized to laryngeal mucosa (see Table 1; Jetté et al., 2020; Soda & Yamamoto, 2012; Takahashi et al., 2016), with functional roles only recently uncovered. First, researchers used optogenetic stimulation of laryngeal epithelia and found that swallowing was evoked by selective activation of these cells, which suggests epithelial cell-to-neuron communication, as opposed to direct neuron activation. Next, authors utilized P2X2/P2X3 knockout mice, which lack ionotropic adenosine triphosphate receptors, in combination with laryngeal challenge and found that, although responses to force and high salt were normal, responses to acid were diminished and completely eliminated for water. Results indicate the specialized and diverse function of epithelial cells at the mucosa surface—acting as an airway sentinels for detection of certain laryngeal chemical challenges. Signaling and protective potential of airway epithelia result from their highly polarized apicobasal field alongside the integrity of their mediolateral field (i.e., intercellular junctions; Fernandez-Sanchez et al., 2015; Levendoski et al., 2014). Laryngeal mucosa exhibits diffuse innervation of intraepithelial nerve fibers (PGP9.5, CGRP, and SP; Corcoran et al., 1999; Domeij et al., 1991; Hisa et al., 1992; Jetté et al., 2020; Kawasoe et al., 1990; Lima-Rodrigues et al., 2004; Matsuo & Shin, 1994; Shin et al., 1987; Takahashi et al., 2016; Tanaka et al., 1993), with putative signaling to neighboring epithelia. Interestingly, CGRP and SP nerve fibers in the rat were found to extend into the lumen and may be exposed and thus receive direct stimulation from irritants and mechanical stimuli at the mucosa–airway interface (Lima-Rodrigues et al., 2004). Noxious stimuli to the larynx activate nerve fibers expressing SP and/or CGPR neuropeptides, thought to play a role in nociception and peripheral neurogenic inflammation via promoting vascular permeability and vasodilatation, respectively—leading to plasma extravasation (Hoyer & Bartfai, 2012; Walker et al., 2010). In the absence of a mucus protective sheath to the epithelium, it has been suggested that exposed nerve endings may stimulate cough reflex and/or neurogenic inflammation (Lima-Rodrigues et al., 2004). This emphasizes the importance of direct activation or manipulation of primary afferents at the mucosa–airway interface for innovating future treatment modalities.

While comprehensive review of mechano-gated ion channels in sensory systems can be found elsewhere (Del Valle et al., 2012; Delmas & Coste, 2013), it is worth discussing the family of nonselective calcium mechano-gated ion channels, PIEZO1 and PIEZO2. Piezo proteins have been extensively studied in recent work and have been deemed essential for development and homeostasis of various tissues that undergo mechanical stress such as lung, skin, cartilage, bladder, vasculature, and heart (Anderson et al., 2017; Cahalan et al., 2015; Li et al., 2014; Nonomura et al., 2017; Ranade et al., 2014; Retailleau et al., 2015; Servin-Vences et al., 2017; Woo et al., 2014). In particular, *Piezo1* has been found to regulate the life cycle of epithelial cells (Eisenhoffer et al., 2012; Gudipaty et al., 2017), while *Piezo2* has been localized to nodose ganglia, vital for establishing efficient respiration at birth and maintaining normal breathing in adults (Nonomura et al., 2017). In addition, vagal *Piezo2* neurons in the mouse were found to mediate airway stretch-induced apnea but did not evoke swallowing expiratory reflexes and/or associated transient apnea under lighter anesthesia (Prescott et al., 2020). Other work has demonstrated *Piezo1* activation results in nuclear shrinkage, thereby regulating chromatin condensation in a Ca^{2+}-dependent manner (Jetta et al., 2019). Furthermore, *Piezo1-YAP* crosstalk has been elucidated in the context of human neural stem cell directed differentiation (Pathak et al., 2014) and proposed as a model for regulation of VF epithelial and mesenchymal remodeling due to its involvement in response to load-induced VF changes (Lungova et al., 2020). Given these data and the biomechanical environment of the larynx, future work targeting these channel proteins offers an exciting and promising area for investigation.

Mechanoreceptors Implicated in Laryngeal Health and Disease

Somatosensory laryngeal disorders represent a significant clinical cohort and present ubiquitous challenges to treatment outcomes in clinical settings (Jafari et al., 2003; Morice et al., 2014, 2006; Murry et al., 2010); sensory perception, if compromised, can have detrimental effects on organismal health and longevity (Delmas & Coste, 2013; Gendron et al., 2015). Understanding iSLN-mediated afference will provide critical insight into normal and disordered laryngeal behaviors. Many disorders of cough, swallow, and voice implicate laryngopharyngeal sensory pathophysiology mediated by mechanoreceptor afferents in airway mucosa. These clinical conditions have been hypothesized to manifest as sensory distortions characterized as hypersensitivity and/or hyposensitivity (chronic cough, laryngospasm, irritable larynx syndrome, paradoxical vocal fold motion [PVFM] disorders; Aviv et al., 1997; Murry et al., 2010).

Age-Related Anatomical and Sensory Changes Over the Life Span

Mechanoreceptors and mechanoproteins have both been implicated in laryngeal disease states with age-related sensory and anatomical changes over the life span (Aviv et al., 1994; Canning, 2011; Erskine et al., 1993; Groneberg et al., 2004; Kawamura et al., 2004; Mortelliti et al., 1990; Sant'Ambrogio & Widdicombe, 2001; Widdicombe, 1998; Yamamoto et al., 2003). In the developing infant larynx, sensorimotor integrative function and tone are altered with laryngomalacia, as demonstrated by increased sensory thresholds via laryngopharyngeal sensory testing, which correlate to disease severity (Thompson, 2007). Laryngopharyngeal sensory testing thresholds were higher in infants with GERD compared to those without, with laryngomalacia symptom improvement attributed to treatment of underlying GERD and a maturational effect of laryngeal reflexes and the CNS over a 9-month period. Results suggested that changes in the peripheral afferent sensory function of the larynx, in combination with underlying delay to the CNS, may predispose an infant to laryngomalacia compared to nondiseased infants and that GERD with laryngopharyngeal reflux (LPR) is likely the most potentiating factor influencing laryngomalacia symptomology. Progressive attenuation in pharyngeal and supraglottic sensitivity with increased age has also been demonstrated in humans via air pulse stimulation and corroborates observed loss of small myelinated fibers within the iSLN, presumed to be sensory, in subjects over 60 years of age (Aviv et al., 1994; Mortelliti et al., 1990). Age-related laryngeal neural dysfunction has been reported in both efferent and afferent nerves (Erskine et al., 1993; Pontoppidan & Beecher, 1960). For example, it has been demonstrated that elderly patients exhibit reduced LAR reflex against exposure of the upper airways to ammonia vapor (Erskine et al., 1993; Pontoppidan & Beecher, 1960). Patients with laryngeal sensory deficits characterized by an absent LAR have 6.8 times the odds of developing pneumonia as compared to those with a present LAR (Kaneoka et al., 2018). Frequency of upper esophageal sphincter response to air stimulation as evidenced via mucosal perturbation to the posterosuperior glottis was also significantly lower in the elderly compared with that in young subjects (Kawamura et al., 2004). In a rat animal model, aging was associated with reduction of laryngeal sensory and secretomotor terminal nerve endings (Yamamoto et al., 2003). Aged rats exhibited morphological changes and reduction in the number of laminar nerve endings, taste buds and their associated nerves, and intraepithelial free nerve endings. Specifically, density and distribution of PGP9.5, CGRP, and SP fibers within laryngeal epithelium were markedly reduced in 35-month-old, compared to 12- and 24-month-old, rats. Data suggest a relationship between age-related changes in laryngeal neural structures and laryngeal sensory dysfunction, which may be contributing factors to development of dysphagia and aspiration-related pneumonia within disordered and/or aged patient populations.

Chronic Cough

One sensory distortion thought to be related to hypersensitivity of laryngeal afferent receptors in airway mucosa presents clinically as refractory chronic cough. Chronic cough is a debilitating disorder and afflicts roughly 10% of the general population (Morice et al., 2014, 2006). Cough is an essential homeostatic reflex preserving airway patency; however, it can often become dry and unproductive, resulting from enhanced excitability of somatosensory afferent nerve endings in mucosa (O'Connell et al., 1996; Riccio et al., 1996; Undem et al., 2002). This cough variant presents in the clinic and is hypothesized to result from augmented peripheral sensitivity in subjects following upper respiratory infections, gastroesophageal reflux disorders, and/or asthma.

Research in the area of cough is extensive, involving both human and animal models. The guinea pig has emerged as the most frequently utilized animal model for reflexive testing. Guinea pigs offer an ideal size for physical manipulation; can be utilized in both conscious and anesthetized models of cough; and, most importantly, initiate cough to similar stimuli as human subjects (e.g., capsaicin, bradykinin, acid, punctate mechanical stimuli; Canning & Chou, 2009). Rats and mice have not been shown to exhibit a cough reflex (Mazzone, 2005). While cough research dates back to the 1950s, we will highlight current knowledge of afferent nerve subtypes and mechanoproteins most often implicated in cough. Comprehensive overview of sensory receptors regulating cough throughout the unified airways can be sourced elsewhere (Mazzone, 2005; Mazzone & McGovern, 2007).

Dichotomy of afferent subtypes has resulted from their differential sensitivity to anesthesia and responsiveness to different stimuli. Although functional classification is commonly utilized, additional delineation can be based upon origin, location in airways, neurochemistry, and/or electrophysiological properties (Mazzone et al., 2003). Broadly speaking, sensory nerves projecting to the airways and lungs have been functionally classified as either predominately mechanosensitive (i.e., low-threshold mechanosensors) or chemosensitive (i.e., chemosensors, unmyelinated C-fibers, nociceptors), albeit strict dichotomy is not always apparent. Two classic types of low-threshold mechanosensors detailed in the intrapulmonary airways of numerous mammalian species are rapidly adapting receptors (RARs) and slowly adapting receptors (SARs; Mazzone & Undem, 2016; Schelegle & Green, 2001; Widdicombe, 2003). Both subtypes have cell bodies in the nodose ganglia, terminate in intrapulmonary airways and lung parenchyma, are sensitive to various mechanical stimuli with conduction action potentials in the Aβ range, but are generally insensitive to a wide range of chemical stimuli (Mazzone, 2005). There is general consensus of at least two vagal afferent pathways that initiate cough upon activation, extrapulmonary cough receptors (concentrated in larynx and carina of trachea) and bronchopulmonary C-fibers, with cell bodies arising from either nodose or jugular ganglia, respectively (Canning, 2011; Undem et al., 2002). Cough receptors have been argued to represent a unique rapidly adapting afferent subtype,

distinct from the well-defined intrapulmonary RARs and SARs throughout the airways, with a primary function to regulate the productive, involuntary cough reflex in airway defense (Canning et al., 2004). Research indicates that cough receptors are polymodal Aδ fibers and are classified as myelinated, capsaicin-insensitive, acid-sensitive mechanoreceptors. Unlike RARs and SARs, which are sensitive to airway stretch and/or bronchospasm, cough receptors respond solely to punctate mechanical stimuli and rapid changes in pH that readily evoke cough from the trachea or larynx in conscious and anesthetized animals and humans (Canning et al., 2004; Mazzone & McGovern, 2007). On the other hand, bronchopulmonary C-fibers have been described as capsaicin-sensitive nociceptors of both Aδ- and C-fiber types, implicated in the nonproductive refractory chronic cough (Canning et al., 2004). Capsaicin, an active component of chili peppers, is well known to evoke the cough reflex by stimulating capsaicin receptors of sensory nerve endings and is frequently used for measurement of cough sensitivity (O'Connell et al., 1996; Uno et al., 2004). Cough receptors rapidly adapt to mechanical probing and acidification with known roles in regulating cough in both conscious and anesthetized animals and humans. This is in contrast to the C-fiber–dependent cough, which is prevented entirely by anesthesia, selectively responsive to capsaicin, bradykinin, protons, and noxious activators of the cation channels, TRPV1 and TRPA1 (Birrell et al., 2009; Groneberg et al., 2004; G. Zhang et al., 2008).

The subfamilies of TRPVs seem to play important functions in regulation of the airway via their physiological role in epithelia lining the mucosa–airway interface (Hamamoto et al., 2008, 2009). Involvement of TRPV1, TRPV4, and/or TRPA1 has been implicated in lower airways for pathological conditions, such as chronic cough (Birrell et al., 2009; Groneberg et al., 2004), and has been associated with traumatic mucositis-induced pain in oral mucosa (Ito et al., 2017; Yamaguchi et al., 2016). Bronchial airway biopsies from patients symptomatic for chronic persistent cough were found to overexpress TRPV1 localized to airway sensory nerves compared to normal controls following a capsaicin challenge (Groneberg et al., 2004). Furthermore, inhibition of citric acid– and capsaicin-induced cough has been demonstrated with TRPV1 channel drug antagonists in guinea pigs (Leung et al., 2007; Undem & Carr, 2010), while TRPV1- and TRPA1-selective antagonists were able to inhibit spontaneous pain in the mouse model (Ito et al., 2017). Results suggest channel targets as novel noncentralized alternatives for antitussive therapy. While mechanoproteins within the upper airway have remained ill-defined, TRPV1 has been exhibited in laryngeal mucosa of both mouse and human (see Table 1), hypothesized to contribute to laryngeal sensitivity (Hamamoto et al., 2009), albeit with unknown functional roles.

Given that mechanical/chemical polymodal irritant receptors have exhibited differential tussigenic sensitivity dependent upon anatomic location along the airway, comparisons between receptor characteristics may offer greater insight. For example, it was found that the elicitability of cough, in anesthetized dogs, with both mechanical (punctate) and chemical stimulation (citric acid) proved to be significantly higher for the tracheobronchial region than for the larynx (Tatar et al., 1994). Cough intensity was also strongly suppressed when mechanical stimulation followed capsaicin administration into trachea or intravenously, consistent with other work (Canning et al., 2004), and suggests that, in anesthetized animals, capsaicin-sensitive nerve activation is not sufficient for evoking cough from the trachea or larynx. Moreover, absence of mechanically induced tracheobronchial cough during bilateral vagal cooling has been described in feline and dog models (Tatar et al., 1994; Widdicombe, 1954). This implies the important role myelinated vagal afferents (i.e., RARs) play in mediating mechanically induced cough from the tracheobronchial tree. Bilateral vagotomy in anesthetized guinea pigs has been shown to decrease respiratory rate and prevent cough evoked via mechanical stimulation of the larynx. More interesting, denervation to the SLN was without effect on the ability to evoke cough; however, cutting of the RLN abolished coughing induced by electrical and mechanical stimulation to the tracheal and laryngeal mucosa (Canning et al., 2004). Subsequent electrophysiological data revealed 88% of nodose ganglia neurons project to the rostral trachea and larynx via the RLN providing additional support for RLN sensory innervation to the proximal subglottis (Yoshida et al., 2000, 1986). One limitation of this study is that the laryngeal receptive field was not explicitly defined; therefore, locations of laryngeal mucosa probing were unknown. Findings overall implicate the additive effect of various stimulants to airway afferent receptors, likely activating both nodose and jugular ganglia neurons, and suggest loss of these receptors or their connections may be the basis for silent aspiration—a significant clinical problem in dysphagia.

Research to date has differentiated cough and bronchopulmonary C-fiber afferent receptors, aimed to characterize productive versus nonproductive cough phenotypes, respectively, although it is likely that both pathways regulate both variants of cough due to interactions within the airways and centrally within the brainstem (Canning, 2009). For example, neuropeptide-containing chemosensors (e.g., SP) were found to play a permissive, yet nonessential, role in cough evoked by mechanoreceptor stimuli in anesthetized guinea pigs (Mazzone & McGovern, 2007). Research in humans and guinea pigs further implicates chemosensitive afferent input from the nose or esophagus may likewise increase cough sensitivity via centrally interacting mechanisms (Brozmanova et al., 2005; Harding & Richter, 1997; Plevkova et al., 2004). Knowledge regarding voltage- and mechano-gated ion channels at the mucosa–airway interface and within peripheral sensory ganglia is rapidly progressing. Currently available antitussive agents offer negligible benefit over placebo for cough relief (Eccles, 2020), in addition to off-target effects raising ethical concerns (Morice et al., 2019). Nonetheless, insights gained may lead to development of novel peripherally acting antitussive drugs that selectively target C-fiber cough pathways, while preserving the important defensive functions of the cough receptor reflex.

PVFM Disorder

Another aberrant sensory distortion often presenting in the clinic is PVFM disorder, also commonly known as *vocal cord dysfunction* or *episodic paroxysmal laryngospasm* (Denipah et al., 2017). This laryngeal-based disorder is characterized by triggered and episodic dyspnea, resulting in disordered breathing and cough related to involuntary periods of VF adduction during inspiration and/or expiration (Murry et al., 2010). While the underlying pathophysiology remains relatively unknown, contributing etiological factors involve abnormal sensory responses via somatosensory feedback from the larynx. It has been hypothesized that decreased sensory receptor thresholds (i.e., hypersensitivity) may promote laryngospasm to normally benign mechanical and/or chemical stimuli (Sinclair et al., 2017), which may explain exhibited associations between LPR and paroxysmal laryngospasm (Loughlin & Koufman, 1996). Patients presenting with cough and LPR demonstrated sensory compromise of the laryngopharynx by reduced laryngopharyngeal sensitivity (i.e., LAR) to air puff stimuli (Aviv et al., 2000; Phua et al., 2005). Cough and PVFM have historically been considered discrete entities; however, recently, it has been suggested that there may be an underlying link between the two conditions (Vertigan et al., 2006). Approximately 80% of patients with chronic cough exhibit PVFM comorbidity (Newman et al., 1995), which is often the primary symptom (Murry et al., 2004), associated with aberrant laryngeal sensation—mainly thought to be caused by LPR (Murry et al., 2010). Acidic environments, characteristic of gastric reflux, have been shown to compromise epithelial barrier function in porcine VF mucosa via reduced transepithelial resistance (Erickson & Sivasankar, 2010) and may contribute to mucosa-based inflammatory reactions. It has been suggested that cough associated with PVFM may be attributed to reduced mechanosensitivity, resulting from afferent receptors obscured within edematous mucosa leading to hyposensitivity (Murry et al., 2010). However, these conclusions are speculative with no direct correlation between changes in sensory perception thresholds and mechanoreceptor function. Recent animal studies utilizing the rat model indicate mechanisms that may be the basis for such abnormalities (Park et al., 2005; Simonyan et al., 2012). To elicit central mechanisms underlying the development of pathological laryngeal responses, researchers induced trauma and/or inflammation to the VF by injection of saline vehicle or lipopolysaccharide solution, respectively, compared to controls absent of any VF manipulation. Immunoreactivity for c-Fos (marker of neuron excitability) and IL-1β (proinflammatory cytokine) was assessed within brainstem regions. While acute laryngeal trauma and inflammation produced elevated c-Fos response in both sensory and motor nuclei of the brainstem (Park et al., 2005), a follow-up study demonstrated prolonged VF inflammation combined with trauma, but not trauma alone, was correlated with increased c-Fos response in brainstem sensory nuclei, limited to the NTS and intermediate/parvicellular reticular formation (Simonyan et al., 2012). IL-1β data were inconsistent across studies. Of particular interest given this increased c-Fos expression in second-order sensory nuclei in the lipopolysaccharide-treated cohort was the absence of correlated c-Fos expression in first-order neurons of laryngeal afferents in nodose and jugular ganglia (Simonyan et al., 2012). This may be due to rapid excitability and resolution of vagal ganglia neurons, in which detection is expressed only in the acute inflammatory phase, which was not assessed in this work. Future research is warranted to fully elucidate changes in vagal ganglia neurons and how these may correlate to central changes and peripheral mechanisms at the mucosa–airway interface. Collectively, studies demonstrate evidence for amplified central sensory response following laryngeal tissue remodeling events, which may be the basis for recalcitrant hypersensitivity changes occurring in patients with chronic laryngeal inflammation. While changes to laryngeal mucosa are highly suspect in patient cohorts, one cannot rule out behavioral components. Regardless, elucidation of potential biological mechanisms at the mucosa–airway interface will help to clarify human behavioral work and targeted drug development and is a significant area awaiting further investigation.

Conclusions

Understanding laryngeal mechanosensory pathways may provide much needed insight into the pathophysiology of sensory-based laryngeal disorders observed in the clinic. Laryngeal somatosensation (interoception) at the cell and molecular level is still largely unexplored. Research suggests heterologous afferent receptors in laryngeal mucosa with cell bodies extending from both nodose and jugular ganglia, likely with polymodal function, albeit a division of labor is apparent at least for some peripheral sensory neurons. Accordingly, mechanotransduction events at the laryngeal mucosa–airway interface via mechanoproteins result in action potential generation and will yield significant insight into the identity and function of definitive mucosal mechanoreceptors—critical for rapid laryngeal control. Recognizing the diverse laryngeal mucosa sensorium will be an important first step for interpretation of electrophysiological and behavioral data as well as development of new drugs for targeted treatment modalities. The fields of genetics and engineering are rapidly evolving with use of more complex transgenic rodent models, which may aid in translation to humans. Application of advanced basic science techniques (optogenetics, antero- or retrograde cell tracing) paired with enhanced optical resolution (time-resolved, single-cell genomics) may prove invaluable for future research. Understanding sensorimotor integrative function of the larynx, especially during development, will provide vital insight for common pediatric clinical presentations (congenital laryngomalacia, idiopathic VF paralysis), assumed, in part, to result from inherited neuropathies. Prevention and treatment of disorders resulting from laryngeal sensory dysfunction is a significant and exciting area awaiting further investigation.

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References

Albegger, K., Hauser-Kronberger, C. E., Saria, A., Graf, A.-H., Bernatzky, G., & Hacker, G. W. (1991). Regulatory peptides and general neuroendocrine markers in human nasal mucosa, soft palate and larynx. *Acta Oto-Laryngologica, 111*(2), 373–378. https://doi.org/10.3109/00016489109137404

Althaus, M., Bogdan, R., Clauss, W. G., & Fronius, M. (2007). Mechano-sensitivity of epithelial sodium channels (ENaCs): Laminar shear stress increases ion channel open probability. *The FASEB Journal, 21*(10), 2389–2399. https://doi.org/10.1096/fj.06-7694com

Alvarez-Berdugo, D., Rofes, L., Casamitjana, J. F., Padrón, A., Quer, M., & Clavé, P. (2016). Oropharyngeal and laryngeal sensory innervation in the pathophysiology of swallowing disorders and sensory stimulation treatments. *Annals of the New York Academy of Sciences, 1380*(1), 104–120. https://doi.org/10.1111/nyas.13150

Anderson, E. O., Schneider, E. R., & Bagriantsev, S. N. (2017). Piezo2 in cutaneous and proprioceptive mechanotransduction in vertebrates. *Current Topics in Membranes, 79,* 197–217. https://doi.org/10.1016/bs.ctm.2016.11.002

Andreatta, R. D., Mann, E. A., Poletto, C. J., & Ludlow, C. L. (2002). Mucosal afferents mediate laryngeal adductor responses in the cat. *Journal of Applied Physiology, 93*(5), 1622–1629. https://doi.org/10.1152/japplphysiol.00417.2002

Aviv, J. E., Liu, H., Parides, M., Kaplan, S. T., & Close, L. G. (2000). Laryngopharyngeal sensory deficits in patients with laryngopharyngeal reflux and dysphagia. *Annals of Otology, Rhinology & Laryngology, 109*(11), 1000–1006. https://doi.org/10.1177/000348940010901103

Aviv, J. E., Martin, J. H., Jones, M. E., Wee, T. A., Diamond, B., Keen, M. S., & Blitzer, A. (1994). Age-related changes in pharyngeal and supraglottic sensation. *Annals of Otology, Rhinology & Laryngology, 103*(10), 749–752. https://doi.org/10.1177/000348949410301001

Aviv, J. E., Sacco, R. L., Thomson, J., Tandon, R., Diamond, B., Martin, J. H., & Close, L. G. (1997). Silent laryngopharyngeal sensory deficits after stroke. *Annals of Otology, Rhinology & Laryngology, 106*(2), 87–93. https://doi.org/10.1177/000348949710600201

Barkmeier, J. M., Bielamowicz, S., Takeda, N., & Ludlow, C. L. (2000). Modulation of laryngeal responses to superior laryngeal nerve stimulation by volitional swallowing in awake humans. *Journal of Neurophysiology, 83*(3), 1264–1272. https://doi.org/10.1152/jn.2000.83.3.1264

Bhabu, P., Poletto, C., Mann, E., Bielamowicz, S., & Ludlow, C. L. (2003). Thyroarytenoid muscle responses to air pressure stimulation of the laryngeal mucosa in humans. *Annals of Otology, Rhinology & Laryngology, 112*(10), 834–840. https://doi.org/10.1177/000348940311201002

Bianconi, R., & Molinari, G. (1962). Electroneurographic evidence of muscle spindles and other sensory endings in the intrinsic laryngeal muscles of the cat. *Acta Oto-Laryngologica, 55*(1–6), 253–259. https://doi.org/10.3109/00016486209127360

Birling, M. C., Herault, Y., & Pavlovic, G. (2017). Modeling human disease in rodents by CRISPR/Cas9 genome editing. *Mammalian Genome, 28*(7–8), 291–301. https://doi.org/10.1007/s00335-017-9703-x

Birrell, M. A., Belvisi, M. G., Grace, M., Sadofsky, L., Faruqi, S., Hele, D. J., Maher, S. A., Freund-Michel, V., & Morice, A. H. (2009). TRPA1 agonists evoke coughing in guinea pig and human volunteers. *American Journal of Respiratory and Critical Care Medicine, 180*(11), 1042–1047. https://doi.org/10.1164/rccm.200905-0665OC

Boushey, H. A., Richardson, P. S., Widdicombe, J. G., & Wise, J. C. M. (1974). The response of laryngeal afferent fibres to mechanical and chemical stimuli. *The Journal of Physiology, 240*(1), 153–175. https://doi.org/10.1113/jphysiol.1974.sp010605

Bradley, R. M. (2000). Sensory receptors of the larynx. *The American Journal of Medicine, 108*(4, Suppl. 1), 47–50. https://doi.org/10.1016/s0002-9343(99)00339-3

Brozmanova, M., Plevkova, J., Bartos, V., Plank, L., & Tatar, M. (2005). Antileukotriene treatment and allergic rhinitis–related cough in guinea pigs. *Journal of Physiology and Pharmacology, 56*(Suppl. 4), 21–30. https://www.ncbi.nlm.nih.gov/pubmed/16204773

Button, B., Okada, S. F., Frederick, C. B., Thelin, W. R., & Boucher, R. C. (2013). Mechanosensitive ATP release maintains proper mucus hydration of airways. *Science Signaling, 6*(279), ra46. https://doi.org/10.1126/scisignal.2003755

Cahalan, S. M., Lukacs, V., Ranade, S. S., Chien, S., Bandell, M., & Patapoutian, A. (2015). Piezo1 links mechanical forces to red blood cell volume. *eLife, 4,* Article e07370. https://doi.org/10.7554/eLife.07370

Canning, B. J. (2009). Central regulation of the cough reflex: Therapeutic implications. *Pulmonary Pharmacology & Therapeutics, 22*(2), 75–81. https://doi.org/10.1016/j.pupt.2009.01.003

Canning, B. J. (2011). Functional implications of the multiple afferent pathways regulating cough. *Pulmonary Pharmacology & Therapeutics, 24*(3), 295–299. https://doi.org/10.1016/j.pupt.2011.01.008

Canning, B. J., & Chou, Y. L. (2009). Cough sensors. I. Physiological and pharmacological properties of the afferent nerves regulating cough. In K. F. Chung & J. Widdicombe (Eds.), *Pharmacology and therapeutics of cough. Handbook of experimental pharmacology* (Vol. 187, pp. 23–47). Springer. https://doi.org/10.1007/978-3-540-79842-2_2.

Canning, B. J., Mazzone, S. B., Meeker, S. N., Mori, N., Reynolds, S. M., & Undem, B. J. (2004). Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea-pigs. *The Journal of Physiology, 557*(2), 543–558. https://doi.org/10.1113/jphysiol.2003.057885

Corcoran, B. M., Jarvis, S., Hahn, C. N., & Mayhew, I. G. (1999). The distribution of nerve fibres immunoreactive for vasoactive intestinal peptide, calcitonin gene-related peptide, substance P and dopamine beta-hydroxylase in the normal equine larynx. *Research in Veterinary Science, 67*(3), 251–259. https://doi.org/10.1053/rvsc.1999.0325

Davis, P. J., & Nail, B. S. (1987). Quantitative analysis of laryngeal mechanosensitivity in the cat and rabbit. *The Journal of Physiology, 388,* 467–485. https://doi.org/10.1113/jphysiol.1987.sp016625

de Carlos, F., Cobo, J., Macías, E., Feito, J., Cobo, T., Calavia, M. G., García-Suárez, O., & Vega, J. A. (2013). The sensory innervation of the human pharynx: Searching for mechanoreceptors. *The Anatomical Record, 296*(11), 1735–1746. https://doi.org/10.1002/ar.22792

Del Valle, M. E., Cobo, T., Cobo, J. L., & Vega, J. A. (2012). Mechanosensory neurons, cutaneous mechanoreceptors, and putative mechanoproteins. *Microscopy Research & Technique, 75*(8), 1033–1043. https://doi.org/10.1002/jemt.22028

Delmas, P., & Coste, B. (2013). Mechano-gated ion channels in sensory systems. *Cell, 155*(2), 278–284. https://doi.org/10.1016/j.cell.2013.09.026

Denipah, N., Dominguez, C. M., Kraai, E. P., Kraai, T. L., Leos, P., & Braude, D. (2017). Acute management of paradoxical vocal fold motion (vocal cord dysfunction). *Annals of Emergency Medicine, 69*(1), 18–23. https://doi.org/10.1016/j.annemergmed.2016.06.045

Domeij, S., Dahlqvist, Å., & Forsgren, S. (1991). Regional differences in the distribution of nerve fibers showing substance P- and calcitonin gene-related peptide-like immunoreactivity in the rat larynx. *Anatomy and Embryology, 183*(1), 49–56. https://doi.org/10.1007/bf00185834

Eccles, R. (2020). The powerful placebo effect in cough: Relevance to treatment and clinical trials. *Lung, 198*(1), 13–21. https://doi.org/10.1007/s00408-019-00305-5

Eisenhoffer, G. T., Loftus, P. D., Yoshigi, M., Otsuna, H., Chien, C. B., Morcos, P. A., & Rosenblatt, J. (2012). Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. *Nature, 484*(7395), 546–549. https://doi.org/10.1038/nature10999

Ellenbroek, B., & Youn, J. (2016). Rodent models in neuroscience research: Is it a rat race? *Disease Models & Mechanisms, 9*(10), 1079–1087. https://doi.org/10.1242/dmm.026120

Erickson, E., & Sivasankar, M. (2010). Simulated reflux decreases vocal fold epithelial barrier resistance. *The Laryngoscope, 120*(8), 1569–1575. https://doi.org/10.1002/lary.20983

Erskine, R. J., Murphy, P. J., Langton, J. A., & Smith, G. (1993). Effect of age on the sensitivity of upper airway reflexes. *British Journal of Anaesthesia, 70*(5), 574–575. https://doi.org/10.1093/bja/70.5.574

Esaki, H., Umezaki, T., Takagi, S., & Shin, T. (1997). Characteristics of laryngeal receptors analyzed by presynaptic recording from the cat medulla oblongata. *Auris Nasus Larynx, 24*(1), 73–83. https://doi.org/10.1016/S0385-8146(96)00015-6

Esteban, A. (1999). A neurophysiological approach to brainstem reflexes. Blink reflex. *Clinical Neurophysiology, 29*(1), 7–38. https://doi.org/10.1016/S0987-7053(99)80039-2

Fernandez-Sanchez, M.-E., Brunet, T., Röper, J.-C., & Farge, E. (2015). Mechanotransduction's impact on animal development, evolution, and tumorigenesis. *Annual Review of Cell and Developmental Biology, 31,* 373–397. https://doi.org/10.1146/annurev-cellbio-102314-112441

Fisher, K. V., Telser, A., Phillips, J. E., & Yeates, D. B. (2001). Regulation of vocal fold transepithelial water fluxes. *Journal of Applied Physiology, 91*(3), 1401–1411. https://doi.org/10.1152/jappl.2001.91.3.1401

Gendron, C. M., Chung, B. Y., & Pletcher, S. D. (2015). The sensory system: More than just a window to the external world. *Communicative & Integrative Biology, 8*(2), Article e1017159. https://doi.org/10.1080/19420889.2015.1017159

Gonçalves da Silva Leite, J., Costa Cavalcante, M. L., Fechine-Jamacaru, F. V., de Lima Pompeu, M. M., Leite, J. A., Nascimento Coelho, D. M., & Rabelo de Freitas, M. (2016). Morphology of nerve endings in vocal fold of human newborn. *International Journal of Pediatric Otorhinolaryngology, 89,* 55–59. https://doi.org/10.1016/j.ijporl.2016.07.020

Groneberg, D. A., Niimi, A., Dinh, Q. T., Cosio, B., Hew, M., Fischer, A., & Chung, K. F. (2004). Increased expression of transient receptor potential vanilloid-1 in airway nerves of chronic cough. *American Journal of Respiratory and Critical Care Medicine, 170*(12), 1276–1280. https://doi.org/10.1164/rccm.200402-174OC

Gudipaty, S. A., Lindblom, J., Loftus, P. D., Redd, M. J., Edes, K., Davey, C. F., Krishnegowda, V., & Rosenblatt, J. (2017). Mechanical stretch triggers rapid epithelial cell division through Piezo1. *Nature, 543*(7643), 118–121. https://doi.org/10.1038/nature21407

Hamamoto, T., Takumida, M., Hirakawa, K., Takeno, S., & Tatsukawa, T. (2008). Localization of transient receptor potential channel vanilloid subfamilies in the mouse larynx. *Acta Oto-Laryngologica, 128*(6), 685–693. https://doi.org/10.1080/00016480701669489

Hamamoto, T., Takumida, M., Hirakawa, K., Tatsukawa, T., & Ishibashi, T. (2009). Localization of transient receptor potential vanilloid (TRPV) in the human larynx. *Acta Oto-Laryngologica, 129*(5), 560–568. https://doi.org/10.1080/00016480802273108

Hammer, M. J., & Krueger, M. A. (2014). Voice-related modulation of mechanosensory detection thresholds in the human larynx. *Experimental Brain Research, 232*(1), 13–20. https://doi.org/10.1007/s00221-013-3703-1

Harding, S. M., & Richter, J. E. (1997). The role of gastroesophageal reflux in chronic cough and asthma. *Chest, 111*(5), 1389–1402. https://doi.org/10.1378/chest.111.5.1389

Hayakawa, T., Kuwahara-Otani, S., Maeda, S., Tanaka, K., & Seki, M. (2014). Calcitonin gene-related peptide immunoreactive sensory neurons in the vagal and glossopharyngeal ganglia innervating the larynx of the rat. *Journal of Chemical Neuroanatomy, 55,* 18–23. https://doi.org/10.1016/j.jchemneu.2013.11.001

Henriquez, V. M., Schulz, G. M., Bielamowicz, S., & Ludlow, C. L. (2007). Laryngeal reflex responses are not modulated during human voice and respiratory tasks. *The Journal of Physiology, 585*(3), 779–789. https://doi.org/10.1113/jphysiol.2007.143438

Henry, B. M., Pękala, P. A., Sanna, B., Vikse, J., Sanna, S., Saganiak, K., Tomaszewska, I. M., Tubbs, R. S., & Tomaszewski, K. A. (2017). The anastomoses of the recurrent laryngeal nerve in the larynx: A meta-analysis and systematic review. *Journal of Voice, 31*(4), 495–503. https://doi.org/10.1016/j.jvoice.2016.11.004

Hisa, Y., Uno, T., Tadaki, N., Murakami, Y., Okamura, H., & Ibata, Y. (1992). Distribution of calcitonin gene-related peptide nerve fibers in the canine larynx. *European Archives of Oto-Rhino-Laryngology, 249*(1), 52–55. https://doi.org/10.1007/bf00175672

Hoyer, D., & Bartfai, T. (2012). Neuropeptides and neuropeptide receptors: Drug targets, and peptide and non-peptide ligands: A tribute to Prof. Dieter Seebach. *Chemistry & Biodiversity, 9*(11), 2367–2387. https://doi.org/10.1002/cbdv.201200288

Hsu, A. K., Rosow, D. E., Wallerstein, R. J., & April, M. M. (2015). Familial congenital bilateral vocal fold paralysis: A novel gene translocation. *International Journal of Pediatric Otorhinolaryngology, 79*(3), 323–327. https://doi.org/10.1016/j.ijporl.2014.12.009

Ito, M., Ono, K., Hitomi, S., Nodai, T., Sago, T., Yamaguchi, K., Harano, N., Gunnjigake, K., Hosokawa, R., Kawamoto, T., & Inenaga, K. (2017). Prostanoid-dependent spontaneous pain and PAR_2-dependent mechanical allodynia following oral mucosal trauma: Involvement of TRPV1, TRPA1, and TRPV4. *Molecular Pain, 13,* 1744806917704138. https://doi.org/10.1177/1744806917704138

Jaalouk, D. E., & Lammerding, J. (2009). Mechanotransduction gone awry. *Nature Reviews Molecular Cell Biology, 10*(1), 63–73. https://doi.org/10.1038/nrm2597

Jafari, S., Prince, R. A., Kim, D. Y., & Paydarfar, D. (2003). Sensory regulation of swallowing and airway protection: A role for the internal superior laryngeal nerve in humans. *The Journal of Physiology, 550*(1), 287–304. https://doi.org/10.1113/jphysiol.2003.039966

Jammes, Y., Fornaris, E., Mei, N., & Barrat, E. (1982). Afferent and efferent components of the bronchial vagal branches in cats. *Journal of the Autonomic Nervous System, 5*(2), 165–176. https://doi.org/10.1016/0165-1838(82)90037-6

Jetta, D., Gottlieb, P. A., Verma, D., Sachs, F., & Hua, S. Z. (2019). Shear stress-induced nuclear shrinkage through activation of Piezo1 channels in epithelial cells. *Journal of Cell Science, 132*(11), jcs226076. https://doi.org/10.1242/jcs.226076

Jetté, M. E., Clary, M. S., Prager, J. D., & Finger, T. E. (2020). Chemical receptors of the arytenoid: A comparison of human and mouse. *The Laryngoscope, 130*(2), 423–430. https://doi.org/10.1002/lary.27931

Jürgens, U. (2002). Neural pathways underlying vocal control. *Neuroscience & Biobehavioral Reviews, 26*(2), 235–258. https://doi.org/10.1016/s0149-7634(01)00068-9

Jürgens, U. (2009). The neural control of vocalization in mammals: A review. *Journal of Voice, 23*(1), 1–10. https://doi.org/10.1016/j.jvoice.2007.07.005

Kaneoka, A., Pisegna, J. M., Inokuchi, H., Ueha, R., Goto, T., Nito, T., Stepp, C. E., LaValley, M. P., Haga, N., & Langmore, S. E. (2018). Relationship between laryngeal sensory deficits, aspiration, and pneumonia in patients with dysphagia. *Dysphagia, 33*(2), 192–199. https://doi.org/10.1007/s00455-017-9845-8

Katta, S., Krieg, M., & Goodman, M. B. (2015). Feeling force: Physical and physiological principles enabling sensory mechanotransduction. *Annual Review of Cell and Developmental Biology, 31,* 347–371. https://doi.org/10.1146/annurev-cellbio-100913-013426

Kawamura, O., Easterling, C., Aslam, M., Rittmann, T., Hofmann, C., & Shaker, R. (2004). Laryngo-upper esophageal sphincter contractile reflex in humans deteriorates with age. *Gastroenterology, 127*(1), 57–64. https://doi.org/10.1053/j.gastro.2004.03.065

Kawasoe, M., Shin, T., & Masuko, S. (1990). Distribution of neuropeptide-like immunoreactive nerve fibers in the canine larynx. *Otolaryngology—Head & Neck Surgery, 103*(6), 957–962. https://doi.org/10.1177/019459989010300612

Kearney, P. R., Mann, E. A., Poletto, C. J., & Ludlow, C. L. (2005). Suppression of thyroarytenoid muscle responses during repeated air pressure stimulation of the laryngeal mucosa in awake humans. *Annals of Otology, Rhinology & Laryngology, 114*(4), 264–270. https://doi.org/10.1177/000348940511400403

Kichko, T. I., Kobal, G., & Reeh, P. W. (2015). Cigarette smoke has sensory effects through nicotinic and TRPA1 but not TRPV1 receptors on the isolated mouse trachea and larynx. *American Journal of Physiology—Lung Cellular and Molecular Physiology, 309*(8), L812–L820. https://doi.org/10.1152/ajplung.00164.2015

Kim, E. J., Jacobs, M. W., Ito-Cole, T., & Callaway, E. M. (2016). Improved monosynaptic neural circuit tracing using engineered rabies virus glycoproteins. *Cell Reports, 15*(4), 692–699. https://doi.org/10.1016/j.celrep.2016.03.067

Landouré, G., Sullivan, J. M., Johnson, J. O., Munns, C. H., Shi, Y., Diallo, O., Gibbs, J. R., Gaudet, R., Ludlow, C. L., Fischbeck, K. H., Traynor, B. J., Burnett, B. G., & Sumner, C. J. (2012). Exome sequencing identifies a novel TRPV4 mutation in a CMT2C family. *Neurology, 79*(2), 192–194. https://doi.org/10.1212/WNL.0b013e31825f04b2

Landouré, G., Zdebik, A. A., Martinez, T. L., Burnett, B. G., Stanescu, H. C., Inada, H., Shi, Y., Taye, A. A., Kong, L., Munns, C. H., Choo, S. S., Phelps, C. B., Paudel, R., Houlden, H., Ludlow, C. L., Caterina, M. J., Gaudet, R., Kleta, R., Fischbeck, K. H., & Sumner, C. J. (2010). Mutations in TRPV4 cause Charcot-Marie-Tooth disease type 2C. *Nature Genetics, 42*(2), 170–174. https://doi.org/10.1038/ng.512

Leung, S. Y., Niimi, A., Williams, A. S., Nath, P., Blanc, F. X., Dinh, Q. T., & Chung, K. F. (2007). Inhibition of citric acid- and capsaicin-induced cough by novel TRPV-1 antagonist, V112220, in guinea-pig. *Cough, 3,* Article 10. https://doi.org/10.1186/1745-9974-3-10

Levendoski, E. E., Leydon, C., & Thibeault, S. L. (2014). Vocal fold epithelial barrier in health and injury: A research review. *Journal of Speech, Language, and Hearing Research, 57*(5), 1679–1691. https://doi.org/10.1044/2014_JSLHR-S-13-0283

Leydon, C., Sivasankar, M., Falciglia, D. L., Atkins, C., & Fisher, K. V. (2009). Vocal fold surface hydration: A review. *Journal of Voice, 23*(6), 658–665. https://doi.org/10.1016/j.jvoice.2008.03.010

Li, J., Hou, B., Tumova, S., Muraki, K., Bruns, A., Ludlow, M. J., Sedo, A., Hyman, A. J., McKeown, L., Young, R. S., Yuldasheva, N. Y., Majeed, Y., Wilson, L. A., Rode, B., Bailey, M. A., Kim, H. R., Fu, Z., Carter, D. A., Bilton, J., . . . Beech, D. J. (2014). Piezo1 integration of vascular architecture with physiological force. *Nature, 515*(7526), 279–282. https://doi.org/10.1038/nature13701

Lima-Rodrigues, M., Nunes, R., & Almeida, A. (2004). Intraepithelial nerve fibers project into the lumen of the larynx. *The Laryngoscope, 114*(6), 1074–1077. https://doi.org/10.1097/00005537-200406000-00022

Loughlin, C. J., & Koufman, J. A. (1996). Paroxysmal laryngospasm secondary to gastroesophageal reflux. *The Laryngoscope, 106*(12), 1502–1505. https://doi.org/10.1097/00005537-199612000-00011

Lu, Y., Ma, X., Sabharwal, R., Snitsarev, V., Morgan, D., Rahmouni, K., Drummond, H. A., Whiteis, C. A., Costa, V., Price, M., Benson, C., Welsh, M. J., Chapleau, M. W., & Abboud, F. M. (2009). The ion channel ASIC2 is required for baroreceptor and autonomic control of the circulation. *Neuron, 64*(6), 885–897. https://doi.org/10.1016/j.neuron.2009.11.007

Ludlow, C. L., Van Pelt, F., & Koda, J. (1992). Characteristics of late responses to superior laryngeal nerve stimulation in humans. *Annals of Otology, Rhinology & Laryngology, 101*(2, Pt. 1), 127–134. https://doi.org/10.1177/000348949210100204

Lungova, V., Griffin, K. V., Lunga, T., & Thibeault, S. L. (2020). Drainage of amniotic fluid delays vocal fold separation and induces load-related vocal fold mucosa remodeling. *Developmental Biology, 466*(1–2), 47–58. https://doi.org/10.1016/j.ydbio.2020.08.003

Mahmoudi, P., Veladi, H., & Pakdel, F. G. (2017). Optogenetics, tools and applications in neurobiology. *Journal of Medical Signals and Sensors, 7*(2), 71–79. https://doi.org/10.4103/2228-7477.205506

Matsuo, H., & Shin, T. (1994). Distribution of intraepithelial nerve fibers in the feline glottis. *Otolaryngology—Head & Neck Surgery, 111*(1), 91–99. https://doi.org/10.1177/019459989411100117

Mazzone, S. B. (2005). An overview of the sensory receptors regulating cough. *Cough, 1,* Article 2. https://doi.org/10.1186/1745-9974-1-2

Mazzone, S. B., Canning, B. J., & Widdicombe, J. G. (2003). Sensory pathways for the cough reflex. In K. F. Chung, J. G. Widdicombe, & H. A. Boushey (Eds.), *Cough: Causes, mechanisms and therapy* (pp. 159–172). Wiley.

Mazzone, S. B., & McGovern, A. E. (2007). Sensory neural targets for the treatment of cough. *Clinical and Experimental Pharmacology and Physiology, 34*(10), 955–962. https://doi.org/10.1111/j.1440-1681.2007.04702.x

Mazzone, S. B., & Undem, B. J. (2016). Vagal afferent innervation of the airways in health and disease. *Physiological Reviews, 96*(3), 975–1024. https://doi.org/10.1152/physrev.00039.2015

Morice, A. H., Jakes, A. D., Faruqi, S., Birring, S. S., McGarvey, L., Canning, B., Smith, J. A., Parker, S. M., Chung, K. F., Lai, K., Pavord, I. D., van den Berg, J., Song, W. J., Millqvist, E., Farrell, M. J., Mazzone, S. B., Dicpinigaitis, P., & Chronic Cough Registry. (2014). A worldwide survey of chronic cough: A manifestation of enhanced somatosensory response. *European Respiratory Journal, 44*(5), 1149–1155. https://doi.org/10.1183/09031936.00217813

Morice, A. H., Kitt, M. M., Ford, A. P., Tershakovec, A. M., Wu, W. C., Brindle, K., Thompson, R., Thackray-Nocera, S., & Wright, C. (2019). The effect of gefapixant, a P2X3 antagonist, on cough reflex sensitivity: A randomised placebo-controlled study. *European Respiratory Journal, 54*(1), 1900439. https://doi.org/10.1183/13993003.00439-2019

Morice, A. H., McGarvey, L., Pavord, I., & British Thoracic Society Cough Guideline Group. (2006). Recommendations for the management of cough in adults. *Thorax, 61*(Suppl. 1), i1–i24. https://doi.org/10.1136/thx.2006.065144

Mortelliti, A. J., Malmgren, L. T., & Gacek, R. R. (1990). Ultrastructural changes with age in the human superior laryngeal nerve. *Archives of Otolaryngology—Head & Neck Surgery, 116*(9), 1062–1069. https://doi.org/10.1001/archotol.1990.01870090078013

Murry, T., Branski, R. C., Yu, K., Cukier-Blaj, S., Duflo, S., & Aviv, J. E. (2010). Laryngeal sensory deficits in patients with chronic cough and paradoxical vocal fold movement disorder. *The Laryngoscope, 120*(8), 1576–1581. https://doi.org/10.1002/lary.20985

Murry, T., Tabaee, A., & Aviv, J. E. (2004). Respiratory retraining of refractory cough and laryngopharyngeal reflux in patients with paradoxical vocal fold movement disorder. *The Laryngoscope, 114*(8), 1341–1345. https://doi.org/10.1097/00005537-200408000-00005

Naidu, L., Lazarus, L., Partab, P., & Satyapal, K. S. (2014). Laryngeal nerve "anastomoses". *Folia Morphologica, 73*(1), 30–36. https://doi.org/10.5603/FM.2014.0005

Naidu, L., Ramsaroop, L., Partab, P., & Satyapal, K. S. (2012). Galen's "anastomosis" revisited. *Clinical Anatomy, 25*(6), 722–728. https://doi.org/10.1002/ca.22011

Newman, K. B., Mason, U. G., III, & Schmaling, K. B. (1995). Clinical features of vocal cord dysfunction. *American Journal of Respiratory and Critical Care Medicine, 152*(4, Pt. 1), 1382–1386. https://doi.org/10.1164/ajrccm.152.4.7551399

Nishijima, K., & Atoji, Y. (2004). Taste buds and nerve fibers in the rat larynx: an ultrastructural and immunohistochemical study. *Archives of Histology and Cytology, 67*(3), 195–209. https://doi.org/10.1679/aohc.67.195

Nonomura, K., Woo, S. H., Chang, R. B., Gillich, A., Qiu, Z., Francisco, A. G., Ranade, S. S., Liberles, S. D., & Patapoutian, A. (2017). Piezo2 senses airway stretch and mediates lung inflation-induced apnoea. *Nature, 541*(7636), 176–181. https://doi.org/10.1038/nature20793

O'Connell, F., Thomas, V. E., Studham, J. M., Pride, N. B., & Fuller, R. W. (1996). Capsaicin cough sensitivity increases during upper respiratory infection. *Respiratory Medicine, 90*(5), 279–286. https://doi.org/10.1016/s0954-6111(96)90099-2

Park, K.-H., Cho, S.-H., Song, C.-E., Kim, D.-H., & Kim, H.-T. (2005). Neuroimmunological activation of the afferent laryngeal neural circuit in experimentally induced laryngeal inflammation. *Acta Oto-Laryngologica, 125*(2), 184–190. https://doi.org/10.1080/00016480410017170

Pathak, M. M., Nourse, J. L., Tran, T., Hwe, J., Arulmoli, J., Le, D. T., Bernardis, E., Flanagan, L. A., & Tombola, F. (2014). Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 111*(45), 16148–16153. https://doi.org/10.1073/pnas.1409802111

Phua, S. Y., McGarvey, L. P. A., Ngu, M. C., & Ing, A. J. (2005). Patients with gastro-oesophageal reflux disease and cough have impaired laryngopharyngeal mechanosensitivity. *Thorax, 60*(6), 488–491. https://doi.org/10.1136/thx.2004.033894

Plevkova, J., Brozmanova, M., Pecova, R., & Tatar, M. (2004). Effects of intranasal capsaicin challenge on cough reflex in healthy human volunteers. *Journal of Physiology and Pharmacology, 55*(Suppl. 3), 101–106. https://www.ncbi.nlm.nih.gov/pubmed/15611600

Pontoppidan, H., & Beecher, H. K. (1960). Progressive loss of protective reflexes in the airway with the advance of age. *JAMA, 174*(18), 2209–2213. https://doi.org/10.1001/jama.1960.03030180029007

Prescott, S. L., Umans, B. D., Williams, E. K., Brust, R. D., & Liberles, S. D. (2020). An airway protection program revealed by sweeping genetic control of vagal afferents. *Cell, 181*(3), 574–589.e14. https://doi.org/10.1016/j.cell.2020.03.004

Puls, I., Jonnakuty, C., LaMonte, B. H., Holzbaur, E. L., Tokito, M., Mann, E., Floeter, M. K., Bidus, K., Drayna, D., Oh, S. J., Brown, R. H., Jr., Ludlow, C. L., & Fischbeck, K. H. (2003). Mutant dynactin in motor neuron disease. *Nature Genetics, 33*(4), 455–456. https://doi.org/10.1038/ng1123

Puls, I., Oh, S. J., Sumner, C. J., Wallace, K. E., Floeter, M. K., Mann, E. A., Kennedy, W. R., Wendelschafer-Crabb, G., Vortmeyer, A., Powers, R., Finnegan, K., Holzbaur, E. L. F., Fischbeck, K. H., & Ludlow, C. L. (2005). Distal spinal and bulbar muscular atrophy caused by dynactin mutation. *Annals of Neurology, 57*(5), 687–694. https://doi.org/10.1002/ana.20468

Qadri, Y. J., Rooj, A. K., & Fuller, C. M. (2012). ENaCs and ASICs as therapeutic targets. *American Journal of Physiology—Cell Physiology, 302*(7), C943–C965. https://doi.org/10.1152/ajpcell.00019.2012

Ranade, S. S., Qiu, Z., Woo, S. H., Hur, S. S., Murthy, S. E., Cahalan, S. M., Xu, J., Mathur, J., Bandell, M., Coste, B., Li, Y. S. J., Chien, S., & Patapoutian, A. (2014). Piezo1, a mechanically activated ion channel, is required for vascular development in mice. *Proceedings of the National Academy of Sciences of the United States of America, 111*(28), 10347–10352. https://doi.org/10.1073/pnas.1409233111

Retailleau, K., Duprat, F., Arhatte, M., Ranade, S. S., Peyronnet, R., Martins, J. R., Jodar, M., Moro, C., Offermanns, S., Feng, Y. Y., Demolombe, S., Patel, A., & Honore, E. (2015). Piezo1 in smooth muscle cells is involved in hypertension-dependent arterial remodeling. *Cell Reports, 13*(6), 1161–1171. https://doi.org/10.1016/j.celrep.2015.09.072

Riccio, M. M., Myers, A. C., & Undem, B. J. (1996). Immunomodulation of afferent neurons in guinea-pig isolated airway. *The Journal of Physiology, 491*(2), 499–509. https://doi.org/10.1113/jphysiol.1996.sp021234

Roudaut, Y., Lonigro, A., Coste, B., Hao, J., Delmas, P., & Crest, M. (2012). Touch sense: Functional organization and molecular determinants of mechanosensitive receptors. *Channels, 6*(4), 234–245. https://doi.org/10.4161/chan.22213

Ruoppolo, G., Schettino, I., Biasiotta, A., Roma, R., Greco, A., Soldo, P., Marcotullio, D., Patella, A., Onesti, E., Ceccanti, M., Albino, F., Giordano, C., Truini, A., De Vincentiis, M., & Inghilleri, M. (2015). Afferent nerve ending density in the human laryngeal mucosa: potential implications on endoscopic evaluation of laryngeal sensitivity. *Dysphagia, 30*(2), 139–144. https://doi.org/10.1007/s00455-014-9589-7

Sampson, S., & Eyzaguirre, C. (1964). Some functional characteristics of mechanoreceptors in the larynx of the cat. *Journal*

of Neurophysiology, 27(3), 464–480. https://doi.org/10.1152/jn.1964.27.3.464

Sanders, I., & Mu, L. (1998). Anatomy of the human internal superior laryngeal nerve. *The Anatomical Record, 252*(4), 646–656. https://doi.org/10.1002/(SICI)1097-0185(199812)252:4<646::AID-AR15>3.0.CO;2-E

Sanders, I., Wu, B. L., Mu, L., Li, Y., & Biller, H. F. (1993). The innervation of the human larynx. *Archives of Otolaryngology—Head & Neck Surgery, 119*(9), 934–939. https://doi.org/10.1001/archotol.1993.01880210022003

Sant'Ambrogio, G., Mathew, O. P., Sant'Ambrogio, F. B., & Fisher, J. T. (1985). Laryngeal cold receptors. *Respiration Physiology, 59*(1), 35–44. https://doi.org/10.1016/0034-5687(85)90016-7

Sant'Ambrogio, G., & Widdicombe, J. (2001). Reflexes from airway rapidly adapting receptors. *Respiration Physiology, 125*(1–2), 33–45. https://doi.org/10.1016/s0034-5687(00)00203-6

Santoso, L. F., Jafari, S., Kim, D. Y., & Paydarfar, D. (2020). The internal superior laryngeal nerve in humans: Evidence for pure sensory function. *The Laryngoscope*. Advance online publication. https://doi.org/10.1002/lary.28642

Sañudo, J. R., Maranillo, E., León, X., Mirapeix, R. M., Orús, C., & Quer, M. (1999). An anatomical study of anastomoses between the laryngeal nerves. *The Laryngoscope, 109*(6), 983–987. https://doi.org/10.1097/00005537-199906000-00026

Sasaki, C. T., Hundal, J. S., & Kim, Y. H. (2005). Protective glottic closure: Biomechanical effects of selective laryngeal denervation. *Annals of Otology, Rhinology & Laryngology, 114*(4), 271–275. https://doi.org/10.1177/000348940511400404

Sasaki, C. T., Jassin, B., Kim, Y. H., Hundal, J., Rosenblatt, W., & Ross, D. A. (2003). Central facilitation of the glottic closure reflex in humans. *Annals of Otology, Rhinology & Laryngology, 112*(4), 293–297. https://doi.org/10.1177/000348940311200401

Sbarbati, A., Merigo, F., Benati, D., Tizzano, M., Bernardi, P., Crescimanno, C., & Osculati, F. (2004). Identification and characterization of a specific sensory epithelium in the rat larynx. *The Journal of Comparative Neurology, 475*(2), 188–201. https://doi.org/10.1002/cne.20172

Schelegle, E. S., & Green, J. F. (2001). An overview of the anatomy and physiology of slowly adapting pulmonary stretch receptors. *Respiration Physiology, 125*(1–2), 17–31. https://doi.org/10.1016/s0034-5687(00)00202-4

Servin-Vences, M. R., Moroni, M., Lewin, G. R., & Poole, K. (2017). Direct measurement of TRPV4 and PIEZO1 activity reveals multiple mechanotransduction pathways in chondrocytes. *eLife, 6,* Article e21074. https://doi.org/10.7554/eLife.21074

Shin, T., Wada, S., Maeyama, T., & Watanabe, S. (1987). Substance P immunoreactive nerve fibers of the canine laryngeal mucosa. *Otolaryngology—Head & Neck Surgery, 97*(1), 39–46. https://doi.org/10.1177/019459988709700107

Simonyan, K., Feng, X., Henriquez, V. M., & Ludlow, C. L. (2012). Combined laryngeal inflammation and trauma mediate long-lasting immunoreactivity response in the brainstem sensory nuclei in the rat. *Frontiers in Integrative Neuroscience, 6,* 97. https://doi.org/10.3389/fnint.2012.00097

Sinclair, C. F., Téllez, M. J., Tapia, O. R., & Ulkatan, S. (2017). Contralateral R1 and R2 components of the laryngeal adductor reflex in humans under general anesthesia. *The Laryngoscope, 127*(12), E443–E448. https://doi.org/10.1002/lary.26744

Soda, Y., & Yamamoto, Y. (2012). Morphology and chemical characteristics of subepithelial laminar nerve endings in the rat epiglottic mucosa. *Histochemistry and Cell Biology, 138*(1), 25–39. https://doi.org/10.1007/s00418-012-0939-y

Stephens, R. E., Wendel, K. H., & Addington, W. R. (1999). Anatomy of the internal branch of the superior laryngeal nerve. *Clinical Anatomy, 12*(2), 79–83. https://doi.org/10.1002/(SICI)1098-2353(1999)12:2<79::AID-CA1>3.0.CO;2-W

Stewart, T. A., & Davis, F. M. (2019). Formation and function of mammalian epithelia: Roles for mechanosensitive PIEZO1 ion channels. *Frontiers in Cell and Developmental Biology, 7,* 260. https://doi.org/10.3389/fcell.2019.00260

Takagi, S., Umezaki, T., & Shin, T. (1995). Convergence of laryngeal afferents with different natures upon cat NTS neurons. *Brain Research Bulletin, 38*(3), 261–268. https://doi.org/10.1016/0361-9230(95)00098-y

Takahashi, N., Nakamuta, N., & Yamamoto, Y. (2016). Morphology of P2X3-immunoreactive nerve endings in the rat laryngeal mucosa. *Histochemistry and Cell Biology, 145*(2), 131–146. https://doi.org/10.1007/s00418-015-1371-x

Tanaka, Y., Yoshida, Y., Hirano, M., Morimoto, M., & Kanaseki, T. (1993). Distribution of SP- and CGRP-immunoreactivity in the cat's larynx. *The Journal of Laryngology & Otology, 107*(6), 522–526. https://doi.org/10.1017/s0022215100123606

Tatar, M., Sant'Ambrogio, G., & Sant'Ambrogio, F. B. (1994). Laryngeal and tracheobronchial cough in anesthetized dogs. *Journal of Applied Physiology (1985), 76*(6), 2672–2679. https://doi.org/10.1152/jappl.1994.76.6.2672

Thompson, D. M. (2007). Abnormal sensorimotor integrative function of the larynx in congenital laryngomalacia: A new theory of etiology. *The Laryngoscope, 117*(Suppl. 114), 1–33. https://doi.org/10.1097/MLG.0b013e31804a5750

Tsuda, K., Maeyama, T., & Shin, T. (1998). Ultrastructure of the myelinated nerve fibers in the feline laryngeal mucosa. *Acta Oto-Laryngologica, 118*(539), 95–97. https://doi.org/10.1080/00016489850182233

Tsujimura, T., Ueha, R., Yoshihara, M., Takei, E., Nagoya, K., Shiraishi, N., Magara, J., & Inoue, M. (2019). Involvement of the epithelial sodium channel in initiation of mechanically evoked swallows in anaesthetized rats. *The Journal of Physiology, 597*(11), 2949–2963. https://doi.org/10.1113/JP277895

Undem, B. J., & Carr, M. J. (2010). Targeting primary afferent nerves for novel antitussive therapy. *Chest, 137*(1), 177–184. https://doi.org/10.1378/chest.09-1960

Undem, B. J., Carr, M. J., & Kollarik, M. (2002). Physiology and plasticity of putative cough fibres in the guinea pig. *Pulmonary Pharmacology & Therapeutics, 15*(3), 193–198. https://doi.org/10.1006/pupt.2002.0362

Uno, T., Koike, S., Bamba, H., Hirota, R., & Hisa, Y. (2004). Capsaicin receptor expression in rat laryngeal innervation. *Annals of Otology, Rhinology & Laryngology, 113*(5), 356–358. https://doi.org/10.1177/000348940411300503

van der Vliet, A., & Bove, P. F. (2011). Purinergic signaling in wound healing and airway remodeling. In M. Picher & R. Boucher (Eds.), *Subcellular biochemistry* (Vol. 55, pp. 139–157). Springer. https://doi.org/10.1007/978-94-007-1217-1_6

Vertigan, A. E., Theodoros, D. G., Gibson, P. G., & Winkworth, A. L. (2006). The relationship between chronic cough and paradoxical vocal fold movement: A review of the literature. *Journal of Voice, 20*(3), 466–480. https://doi.org/10.1016/j.jvoice.2005.08.001

Walker, C. S., Conner, A. C., Poyner, D. R., & Hay, D. L. (2010). Regulation of signal transduction by calcitonin gene-related peptide receptors. *Trends in Pharmacological Sciences, 31*(10), 476–483. https://doi.org/10.1016/j.tips.2010.06.006

Widdicombe, J. G. (1954). Respiratory reflexes from the trachea and bronchi of the cat. *The Journal of Physiology, 123*(1), 55–70. https://doi.org/10.1113/jphysiol.1954.sp005033

Widdicombe, J. G. (1998). Afferent receptors in the airways and cough. *Respiration Physiology, 114*(1), 5–15. https://doi.org/10.1016/s0034-5687(98)00076-0

Widdicombe, J. G. (2003). Functional morphology and physiology of pulmonary rapidly adapting receptors (RARs). *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology, 270*(1), 2–10. https://doi.org/10.1002/ar.a.10003

Woo, S. H., Ranade, S., Weyer, A. D., Dubin, A. E., Baba, Y., Qiu, Z., Petrus, M., Miyamoto, T., Reddy, K., Lumpkin, E. A., Stucky, C. L., & Patapoutian, A. (2014). Piezo2 is required for Merkel-cell mechanotransduction. *Nature, 509*(7502), 622–626. https://doi.org/10.1038/nature13251

Yamaguchi, K., Ono, K., Hitomi, S., Ito, M., Nodai, T., Goto, T., Harano, N., Watanabe, S., Inoue, H., Miyano, K., Uezono, Y., Matoba, M., & Inenaga, K. (2016). Distinct TRPV1- and TRPA1-based mechanisms underlying enhancement of oral ulcerative mucositis-induced pain by 5-fluorouracil. *Pain, 157*(5), 1004–1020. https://doi.org/10.1097/j.pain.0000000000000498

Yamamoto, Y., Atoji, Y., Hobo, S., Yoshihara, T., & Suzuki, Y. (2001). Morphology of the nerve endings in laryngeal mucosa of the horse. *Equine Veterinary Journal, 33*(2), 150–158. https://doi.org/10.1111/j.2042-3306.2001.tb00593.x

Yamamoto, Y., Atoji, Y., Kuramoto, H., & Suzuki, Y. (1998). Calretinin-immunoreactive laminar nerve endings in the laryngeal mucosa of the rat. *Cell & Tissue Research, 292*(3), 613–617. https://doi.org/10.1007/s004410051091

Yamamoto, Y., Atoji, Y., & Suzuki, Y. (2000). Calbindin D28k-immunoreactive afferent nerve endings in the laryngeal mucosa. *The Anatomical Record, 259*(3), 237–247. https://doi.org/10.1002/1097-0185(20000701)259:3<237::AID-AR20>3.0.CO;2-P

Yamamoto, Y., Hosono, I., Atoji, Y., & Suzuki, Y. (1997). Morphological study of the vagal afferent nerve endings in the laryngeal mucosa of the dog. *Annals of Anatomy, 179*(1), 65–73. https://doi.org/10.1016/S0940-9602(97)80138-0

Yamamoto, Y., Tanaka, S., Tsubone, H., Atoji, Y., & Suzuki, Y. (2003). Age-related changes in sensory and secretomotor nerve endings in the larynx of F344/N rat. *Archives of Gerontology and Geriatratrics, 36*(2), 173–183. https://doi.org/10.1016/s0167-4943(02)00165-6

Yamamoto, Y., & Taniguchi, K. (2005). Immunolocalization of VR1 and VRL1 in rat larynx. *Autonomic Neuroscience, 117*(1), 62–65. https://doi.org/10.1016/j.autneu.2004.11.003

Yamamoto, Y., & Taniguchi, K. (2006). Expression of ENaC subunits in sensory nerve endings in the rat larynx. *Neuroscience Letters, 402*(3), 227–232. https://doi.org/10.1016/j.neulet.2006.04.044

Yamashita, T., Nash, E. A., Tanaka, Y., & Ludlow, C. L. (1997). Effects of stimulus intensity on laryngeal long latency responses in awake humans. *Otolaryngology—Head & Neck Surgery, 117*(5), 521–529. https://doi.org/10.1016/s0194-5998(97)70025-1

Yoshida, Y., Tanaka, Y., Hirano, M., & Nakashima, T. (2000). Sensory innervation of the pharynx and larynx. *The American Journal of Medicine, 108*(4, Suppl. 1), 51–61. https://doi.org/10.1016/s0002-9343(99)00342-3

Yoshida, Y., Tanaka, Y., Mitsumasu, T., Hirano, M., & Kanaseki, T. (1986). Peripheral course and intramucosal distribution of the laryngeal sensory nerve fibers of cats. *Brain Research Bulletin, 17*(1), 95–105. https://doi.org/10.1016/0361-9230(86)90165-6

Yoshida, Y., Tanaka, Y., Saito, T., Shimazaki, T., & Hirano, M. (1992). Peripheral nervous system in the larynx: An anatomical study of the motor, sensory and autonomic nerve fibers. *Folia Phoniatrica et Logopaedica, 44*(5), 194–219. https://doi.org/10.1159/000266152

Zeng, W. Z., Marshall, K. L., Min, S., Daou, I., Chapleau, M. W., Abboud, F. M., Liberles, S. D., & Patapoutian, A. (2018). PIEZOs mediate neuronal sensing of blood pressure and the baroreceptor reflex. *Science, 362*(6413), 464–467. https://doi.org/10.1126/science.aau6324

Zhang, G., Lin, R. L., Wiggers, M., Snow, D. M., & Lee, L. Y. (2008). Altered expression of TRPV1 and sensitivity to capsaicin in pulmonary myelinated afferents following chronic airway inflammation in the rat. *The Journal of Physiology, 586*(23), 5771–5786. https://doi.org/10.1113/jphysiol.2008.161042

Zhang, L., Jones, S., Brody, K., Costa, M., & Brookes, S. J. H. (2004). Thermosensitive transient receptor potential channels in vagal afferent neurons of the mouse. *American Journal of Physiology—Gastrointestinal and Liver Physiology, 286*(6), G983–G991. https://doi.org/10.1152/ajpgi.00441.2003

Zimon, M., Baets, J., Auer-Grumbach, M., Berciano, J., Garcia, A., Lopez-Laso, E., Merlini, L., Hilton-Jones, D., McEntagart, M., Crosby, A. H., Barisic, N., Boltshauser, E., Shaw, C. E., Landoure, G., Ludlow, C. L., Gaudet, R., Houlden, H., Reilly, M. M., Fischbeck, K. H., . . . Jonghe, P. D. (2010). Dominant mutations in the cation channel gene *transient receptor potential vanilloid 4* cause an unusual spectrum of neuropathies. *Brain, 133*(6), 1798–1809. https://doi.org/10.1093/brain/awq109