The Laryngoscope

Healthy Human Laryngopharyngeal Sensory Innervation Density Correlates with Age

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Objective: Somatosensory feedback from upper airway structures is essential for swallowing and airway defense but little is known about the identities and distributions of human upper airway neurons. Furthermore, whether sensory innervation modifies with aging is unknown. In this study, we quantify neuronal and chemosensory cell density in upper airway structures and correlate with age.

Methods: Participants underwent biopsies from base of tongue, lateral and midline pharyngeal wall, epiglottis, and arytenoids (N = 25 13 female/12 male; 20–80 years, mean 51.4 years without clinical diagnosis of dysphagia or clinical indication for biopsy). Tissue sections were labeled with antibodies for all neurons, myelinated neurons, and chemosensory cells. Densities of lamina propria innervation, epithelial innervation, solitary chemosensory cells, and taste buds were calculated and correlated with age.

Results: Arytenoid had the highest density of innervation and chemosensory cells across all measures compared to other sites. Taste buds were frequently observed in arytenoid and epiglottis. Base of tongue, lateral pharynx, and midline posterior pharynx had minimal innervation and few chemosensory cells. Epithelial innervation was present primarily in close proximity to chemosensory cells and taste buds. Overall innervation and myelinated fibers in the arytenoid lamina propria decline with aging.

Conclusion: Findings establish the architecture of healthy adult sensory innervation and demonstrate the varied distribution of laryngopharyngeal innervation, necessary steps toward understanding the sensory basis for swallowing and airway defense. We also document age-related decline in arytenoid innervation density. These findings suggest that sensory afferent denervation of the upper airway may be a contributing factor to presbyphagia.

Key Words: dysphagia, somatosensation, presbyphagia, airway defense.

Level of Evidence: NA

***Laryngoscope*, 00:1–12, 2022**

INTRODUCTION

Dysphagia is highly prevalent amongst the elderly and those with neurological disorders and is responsible for significant morbidity, including malnutrition, aspiration pneumonia, asphyxiation, decreased quality of life, and mortality.[1–4] In fact, aspiration pneumonia is a leading cause of death in Parkinson's disease, stroke, amyotrophic lateral sclerosis, Alzheimer's disease, and other neurological diseases.[5–9] Dysphagia is present in 40% of adults over the age of 60 and approximately 50% of individuals in nursing homes.[10–12] Presbyphagia, swallowing dysfunction associated with natural aging in otherwise healthy adults, is also common amongst the elderly.[13,14] Delays in initiation of the pharyngeal phase of swallowing and airway protective reflexes, as well as decrease in pharyngeal pressure during swallow are associated with aging.[12,15,16] Despite the high prevalence of swallowing dysfunction, little is known about the identities of sensory neurons that coordinate human swallowing and whether they are modified with age.[1,17,18]

Several upper airway locations are hypothesized to be innervated by sensory neurons essential for swallowing and airway protection including arytenoids, epiglottis, pharyngeal walls, and base of tongue.[1,17,19–22] During ingestion, these sites contact foods, providing opportunities for initiation of sensory feedback. The tongue and lateral pharyngeal walls contract to propel the bolus into the esophagus.[23] During swallowing, the epiglottis closes over the airway, protecting it from particulate invasion while guiding the bolus toward the esophagus.[24,25] The arytenoids facilitate vocal fold closure

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Additional supporting information may be found in the online version of this article.

Editor's Note: This Manuscript was accepted for publication on June 16, 2022.

Meeting Information: American Broncho-Esophagological Association, Dallas, TX, USA. April 29–May 1, 2022.

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

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DOI: 10.1002/lary.30287

through the laryngeal adductor reflex (LAR), which acts as a secondary prevention measure against particulates entering the airway.[24,25] Protective behaviors such as cough, swallow, and apnea are triggered when laryngopharyngeal sites are stimulated by mechanical, osmotic, chemical, or electrical stimulation emphasizing the importance of somatosensory stimulations to airway defense.[17,19–22,26–30] Molecularly distinct subpopulations of sensory neurons are involved in evoking airway defense reflexes including P2RY1 neurons that evoke apnea, swallowing, and vocal fold adduction in response to water and acid in mice.[17] Though human studies are limited, transient receptor potential (TRP) channels are expressed in neurons of laryngopharyngeal structures of mice and humans.[31–33] TRPV1, TRPA1, and TRPM8 agonists have been found to modulate swallow and are being investigated as potential therapeutics for dysphagia.[27,34–36] Mechanosensory neurons densely populate the arytenoid and epiglottis and consist of large-diameter, myelinated populations of neurons. The majority of these are either silent at rest with rapidly adapting responses to pressure or tonically active with sustained responses to pressure, similar to encapsulated corpuscles or muscle spindles, respectively.[37] Mechanical stimulation of the arytenoid with pressure or airpuff results in LAR in several species, indicating that mechanosensory neurons are likely involved in airway defense.[38,39] The mechanosensory ion channels that mediate swallow and airway defense are not known, but ENaC and Piezo2 are promising candidates.[17,40] Clinically, decreased laryngeal sensation is associated with both silent and clinical aspiration and it was found that sensory loss can lead to dysphagia in the absence of motor dysfunction.[26,41–43] Despite evidence showing the importance of sensation in swallow, little is known about the mechanosensory neurons in the upper airway that coordinate swallow and airway protection in humans, and whether they undergo changes with aging.[17,18,44–46] Understanding sensory substrates of swallow have the potential to inform the development of novel treatments for dysphagia.

Peripheral somatosensory neurons undergo age-related decline across body regions including the oral cavity.[45,47–50] We propose that a similar phenomenon occurs in the laryngopharynx and could contribute to presbyphagia. To test this, we quantified and compared innervation and chemosensory cell density across the laryngopharynx and described the normal complement and morphology of somatosensory innervation in upper airway regions. We assessed correlations between innervation density and age.

MATERIALS & METHODS

Study Design

To analyze somatosensory neurons innervating the healthy human laryngopharynx, we investigated neurochemical and anatomical features of sensory afferents innervating sites involved in the swallow reflex.[1,22] Tissue sections were labeled with immunohistochemical markers for all neurons (βIII-tubulin, βIII), myelinated neuronal afferents (Neurofilament Heavy, NFH), chemosensory cells (Keratin 20, K20), and nuclei (DAPI) allowing for distinction between the majority of myelinated low-threshold mechanosensory neurons (βIII+, NFH+) from unmyelinated chemosensory, thermosensory, and most nociceptive neurons (βIII+, NFH−).[51]

Volunteer Enrollment

Adult volunteers (N = 25, ≥18 years old, 13 female, 12 male) were eligible for enrollment. Participants ranged from 20 to 80 years old (mean age = 51.4 years) with no clinical diagnosis of dysphagia; no neurological or muscular disorders that interfere with pharynx or larynx function; no indication for laryngopharyngeal biopsy; and no history of inflammatory or pain conditions, chemotherapy, and/or radiation treatment. Written informed consent was obtained from all patients prior to any protocol-specific procedures, which were pre-approved by the Columbia University Institutional Review Board.

Tissue Collection, Processing, and Histology

Biopsies were obtained using Olympus Alligator Jaw forceps (FB-15C-1, 5.5 mm cup) from base of tongue (near the lingual tonsil posterior to the circumvallate papillae), lateral and midline pharyngeal wall, tip of epiglottis, and arytenoids. One participant tolerated only 3/5 biopsies. All other participants completed the five biopsies, resulting in 25 tissue samples for arytenoid, epiglottis, and lateral pharyngeal wall; and 24 samples of base of tongue and midline posterior pharyngeal wall. Biopsies were flash frozen in Tissue-Tek OCT and stored at −80°C and analyzed using section immunohistochemistry.

Immunohistochemistry was performed as previously described.[51] Tissue was cryosectioned (Leica Microsystems, Wetzlar, Germany) at 20 or 25 μm thickness on gelatin coated Superfrost slides (Fisherbrand) with ≥100 μm between sections, and then stored at −80°C. Slides were baked for one hour (37°C), and incubated in phosphate buffered saline (PBS) containing 0.1% Triton-X 100 (PBST) and 5% normal goat serum (NGS) at room temperature for one hour. Slides were then incubated overnight (4°C) with primary antibodies diluted in PBST+NGS. Slides were washed for 3 × 5 min in PBST and incubated with secondary antibodies (1:1000) for one hour at room temperature, washed for 5 × 5 min in PBS, mounted in Fluoromount-G with DAPI (Southern Biotech) for nuclear labeling, cover-slipped, sealed with nail polish, and stored at 4°C. See Table S1 for antibody information.

Imaging

Specimens were imaged on a Leica SP8 confocal microscope by an age-blinded evaluator. Z-stack images were taken at 2048 × 2048 pixels using a 20X, NA = 0.75 objective lens at 0.75 zoom with sequential scanning of fluorophores. Slight modifications were made to intensity and gain to accommodate for changes in laser power over time. The middle three sections for each biopsy were

imaged for quantitative analysis of innervation and chemosensory cell densities. If a given section was of insufficient quality (e.g., no lamina propria) that section was skipped and the adjacent section was used for quantification. Samples were excluded from analysis if an insufficient number of acceptable sections were identified. Taste buds were quantified in the entire biopsy. Additional display images were taken using a 40x, NA = 1.30 objective lens and processed using ImageJ, Adobe Photoshop, and Adobe Illustrator. Minor adjustments for brightness, contrast, and threshold were made in Adobe Photoshop and applied to the entire image for qualitative analysis.

Densitometry

Innervation was quantified by an age-blinded evaluator in ImageJ using nerve fiber pixel density (Fig 1A–D). Lamina propria innervation was quantified from the basement membrane to a sub-epithelial depth of 100 μm (Fig 1B). Epithelial quantification included the area from the basement membrane to the superficial edge of the epithelium. Feature J was applied to the raw image (Fig 1A) of βIII and NFH individual channels (Fig 1C) using the smallest hessian eigenvalues for selection of linear structures and converted to a binary image (Fig 1D) using Yen threshold.[52,53] NFH and βIII fibers innervating chemosensory structures were excluded from intra-epithelial innervation density calculations by removing K20 positive areas from calculation to select for putative somatosensory endings. Artifacts, defined by having non-fibrous morphology of homogenous staining present in >1 channel, were excluded from density calculations. Innervation density was calculated as the average % pixilated area for three sections.

Chemosensory cells from three images were quantified and divided by the cumulative epithelial length of those images. All sections were viewed for the presence of taste buds, defined as the tight clustering of ≥3 K20+ cell bodies to differentiate from K20+ solitary chemosensory cells, and imaged when present. The total number of taste buds for a given sample was divided by the total epithelial length. To determine total epithelial length, samples were imaged using fluorescent whole slide imaging (Leica Versa 8, 5x lens, NA = 0.15) then calculated in ImageJ by tracing the basement membrane of all sections.

Quantification and Statistics

Densities were calculated in ImageJ and correlated with age using linear regression in GraphPad Prism 9. Data were tested for normality and Kruskal-Wallis or Brown-Forsythe ANOVA with post-hoc analysis (Dunn's or Dunnett's T3, respectively) were applied as appropriate. Simple linear regression was used to investigate differences in innervation densities with age.

RESULTS

Arytenoids. Decreased arytenoid mechanosensation is correlated with increased penetration and tracheal aspiration.[26] In agreement with previous work, the arytenoid was densely innervated, had abundant taste buds (present in 19/25 biopsies), and numerous chemosensory cells (Fig 2).[54,55] Taste buds were unevenly dispersed within the epithelium and often found in clusters (Fig 2A) with stretches of epithelium entirely lacking taste buds. βIII+, NFH+ and βIII+, NFH- nerve fibers were often found in the lamina propria directly beneath taste buds (Fig 2A–C), with afferents crossing the basement membrane and extending within and adjacent to taste buds (Fig 2B,C). Rare structures resembling end bulbs of Krause were also identified (Fig 2B). Epithelial βIII and NFH immunoreactivity was infrequent except in proximity to K20+ staining, where βIII and NFH were found both intra- and extra-gemmally (Fig 2B,C). Intra-gemmal taste bud innervation was predominantly βIII+, NFH−, and to a lesser extent βIII+, NFH+(Fig 2B,C). Unlike taste buds, solitary chemosensory cells rarely colocalized with βIII+ or NFH+ staining, although they were also found in areas with adjacent epithelial βIII and NFH innervation (Fig 2D,E). Arytenoid sections containing K20+ cells occasionally showed areas of unusually dense epithelial βIII+, NFH− immunoreactivity (Fig 2F,G).

Epiglottis. Mechanical stimulation to the epiglottis induces swallowing,[22] and electrical stimulation can induce the laryngeal adductor reflex.[56] Furthermore, application of chemicals to the epiglottis initiates robust activity in the superior laryngeal nerve.[20] As expected, K20+ chemosensory structures were common in the epiglottis (Fig 3A–E). Taste buds were present in 16/25 biopsies and had similar organization and innervation patterns as arytenoid. Abundant neuronal afferents resided directly beneath taste buds in the lamina propria and intra- and peri-gemmal βIII and NFH staining was common (Fig 3A–D). Rare putative end bulbs of Krause were identified in the epiglottis (Fig 3F,G).

Base of Tongue. Pressure applied to the base of tongue evokes gag reflex; therefore, we expected mechanosensory fibers to be present in this region.[57] Surprisingly, the base of tongue typically had low innervation density (Fig 4A) and taste buds were only observed in 6/23 biopsies (Fig 4B,C). When present, taste buds were associated with intra-gemmal innervation and abundant neuronal afferents in the lamina propria (Fig 4B,C). One biopsy with taste buds had strikingly dense innervation in the lamina propria (Fig 4D–F).

Pharyngeal Wall. Mechanical stimulation of the posterior pharyngeal wall triggers a swallow reflex,[22,57] during which the pharyngeal muscles (including the lateral pharynx) contract in peristalsis, guiding food or liquid toward the esophagus. Thus, we assessed innervation of biopsies from both the lateral pharyngeal wall (Fig 5A-E) and the midline of the posterior pharyngeal wall (Fig 5F-I). Both sites were sparsely innervated (Fig 5A, F) and had few taste buds or chemosensory cells (Fig 5). Only 3/25 biopsies of the lateral pharynx and 2/24 biopsies of the posterior pharynx included taste buds (Fig 5B,C,G,H).

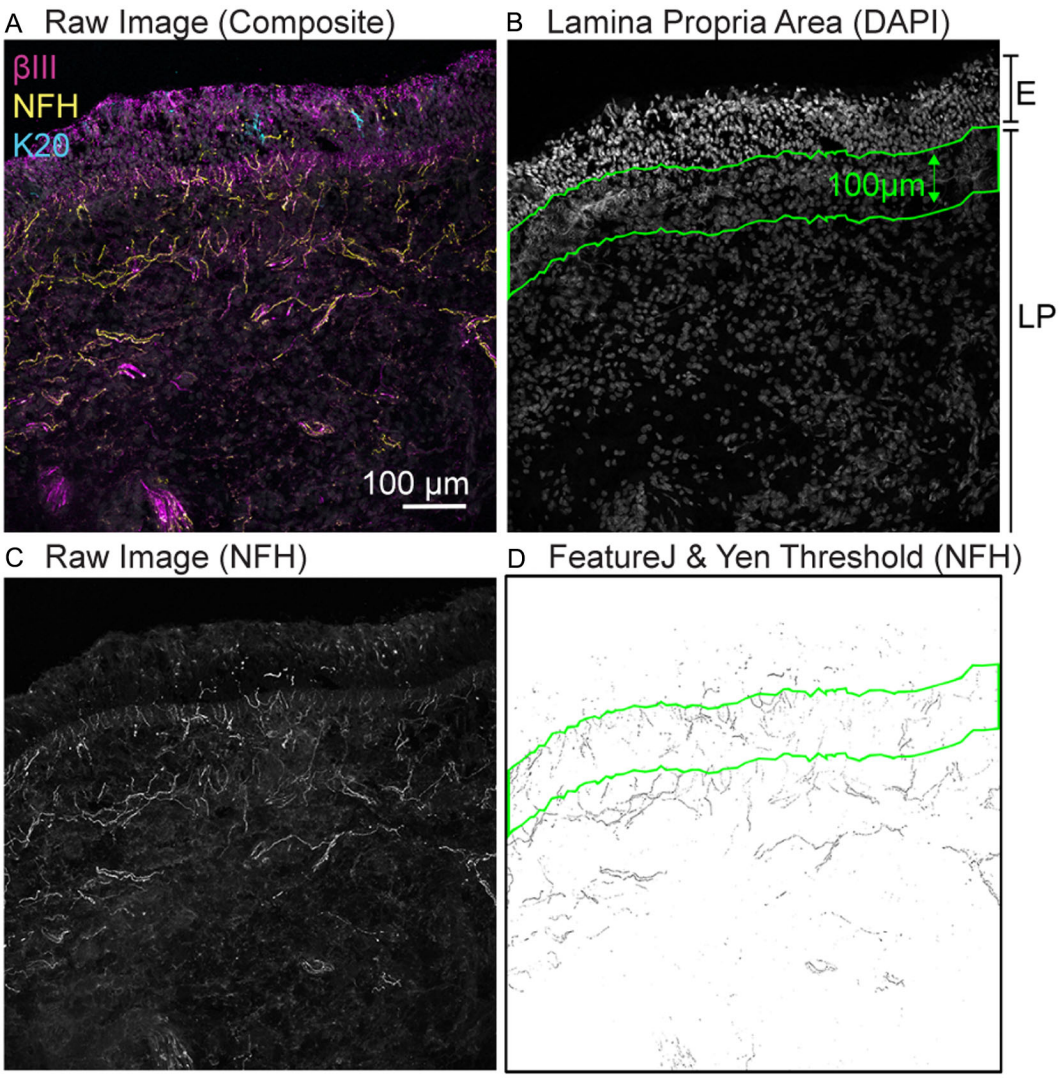

Fig. 1. Pipeline for quantification of βIII and NFH innervation density. (A) Composite image of arytenoid labeled with βIII (magenta), NFH (yellow), and K20 (cyan). (B) Innervation density of the lamina propria was determined using the area 100 μm below the epithelia-lamina propria border as demonstrated by the green outline. DAPI nuclear labeling facilitated identification of epithelia-lamina propria border (Epithelium, E; Lamina Propria, LP). (C) Raw NFH immunoreactivity before threshold was applied for quantification of innervation density. (D) NFH image following application of Feature J to select for the smallest Hessian values, and conversion into a binary image using Yen threshold. Innervation density for the lamina propria was then measured as the density of pixelated area within 100 μm from the basement membrane (green box). [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

When present, taste buds were associated with dense neuronal afferents in both the epithelium and lamina propria, though not to the same extent as arytenoid or epiglottis (Fig 5B,C,G,H). Solitary chemosensory cells were not associated with increased neuronal afferent density (Fig 5D,E).

Quantification of Sensory Structures. We next quantified and compared the density of innervation and chemosensory structures in all tissues (Fig 6, Tables S2–S4). Densitometry was used to quantify and compare innervation of the lamina propria (Fig 1). Arytenoid had significantly higher density of βIII and NFH compared to base of tongue, lateral pharyngeal wall, and midline posterior pharyngeal wall (Fig 6A,B). Epiglottis contained higher NFH+ neuronal density compared to midline posterior pharyngeal wall (Fig 6B). Overall, epithelium had minimal innervation except when K20+ cells were also present. βIII intra-epithelial nerve density did not significantly differ across sites; however, arytenoid had significantly higher NFH epithelial density compared to all other sites (Fig 6C,D). Taste bud density was significantly higher in the arytenoid and epiglottis compared to other sites (Fig 6E). Solitary chemosensory cells were significantly more dense in the arytenoid compared to base of tongue and both pharyngeal locations (Fig 6F).

Given that both sensory acuity and swallowing abilities decline with age, we next asked whether laryngopharyngeal innervation and/or chemosensory structure density experience a similar reduction. Simple linear regression analysis was performed for all

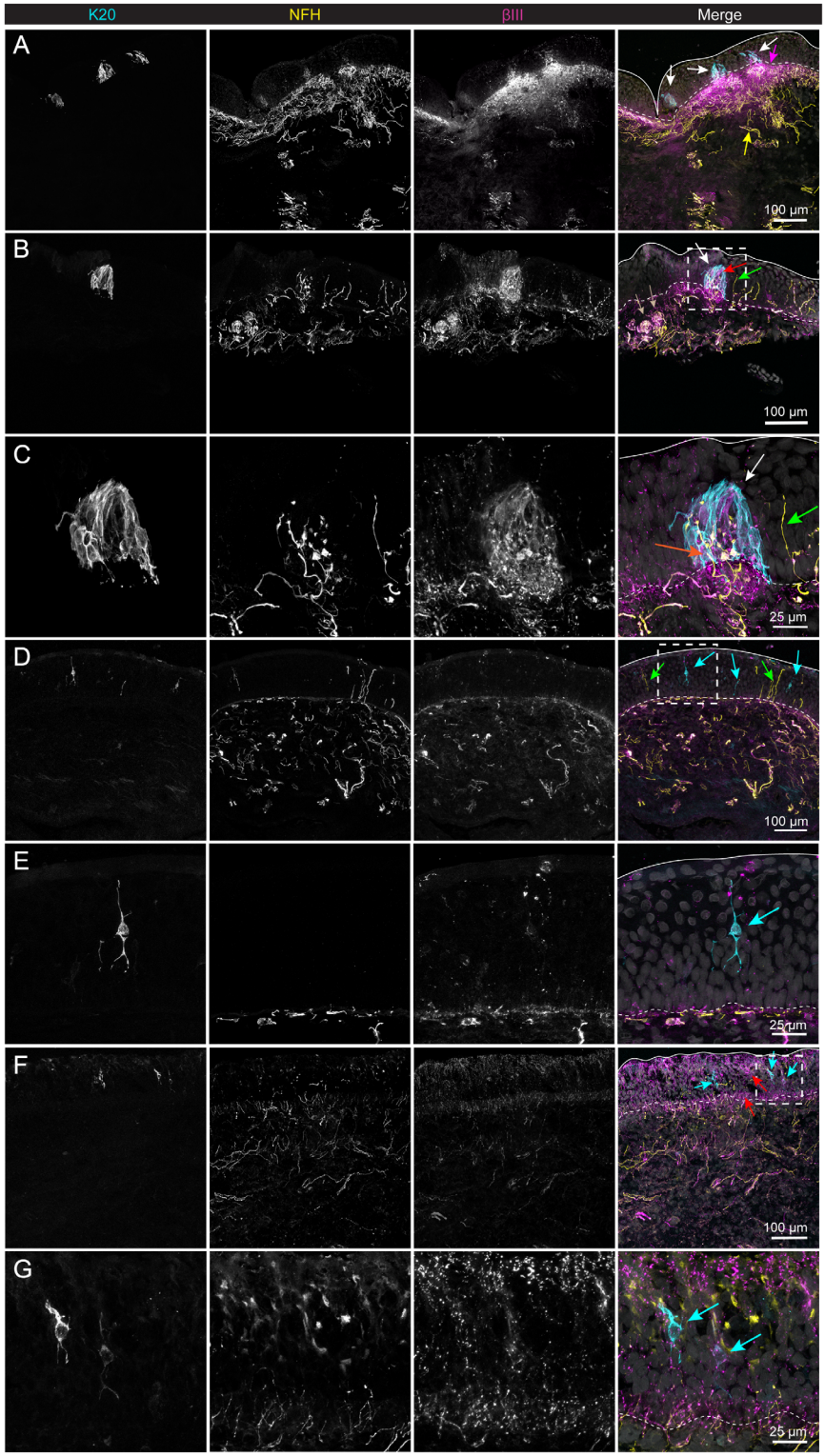

Fig. 2. Arytenoid was densely innervated and had abundant chemosensory structures. Solid lines indicate epithelial surface. Dashed lines indicate epithelial-lamina propria border. (A) Representative image of arytenoid tissue including dense βIII+, NFH− (magenta arrow) and βIII+, NFH+ (yellow arrow) innervation within the lamina propria and frequent taste buds (white arrows). (B) Highly innervated arytenoid tissue demonstrating taste buds (white arrow), intragemmal βIII+, NFH− and βIII+, NFH+ innervation (red arrow) and extragemmal NFH+ innervation (green arrow). Rare putative end bulbs of Krause were also identified (gray arrows). Box indicates region in (C). (C) High magnification image of (B). (D) K20+ solitary chemosensory cells (cyan arrows) were abundant in arytenoid tissue, here shown with interspersed epithelial NFH+ (green arrows) innervation. Box indicates region in (E). (E) High magnification image of (D). (F) Dense epithelial βIII+ (red arrow) innervation was most frequently observed in K20+ (cyan arrows) arytenoid tissue. Box indicates region in (G). (G) High magnification image of (F). [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

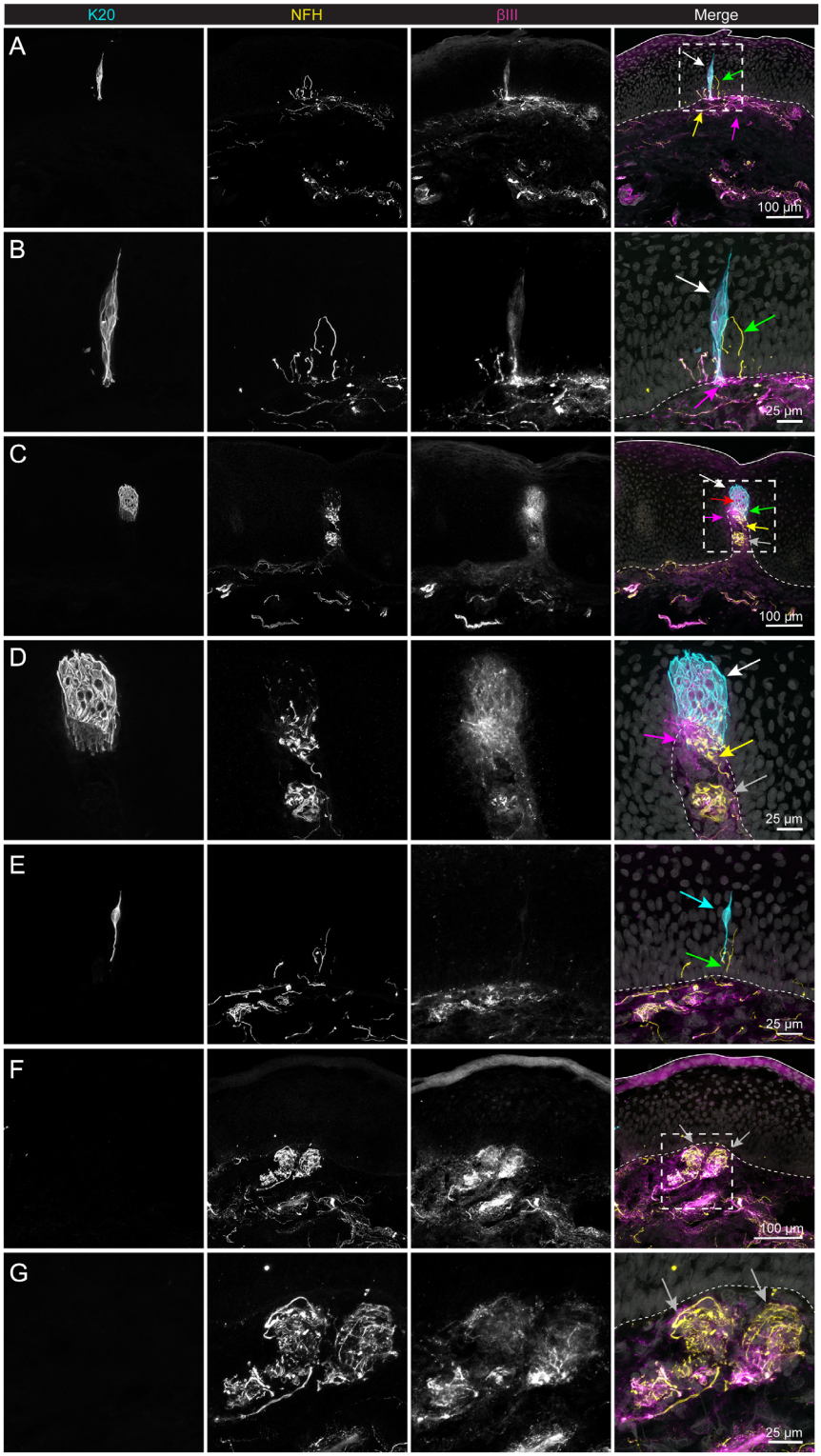

Fig. 3. Epiglottis was densely innervated with βIII+ and NFH+ neurons as well as chemosensory cells. Solid lines indicate epithelial surface. Dashed lines indicate epithelial-lamina propria border. (A) Representative image of epiglottis tissue with frequent K20+ staining (white arrow) and βIII+, NFH− (magenta arrow) and βIII+, NFH+ (yellow arrow) neurons in the lamina propria. Epithelial NFH+ fibers (green arrow) were rarely seen except in proximity to K20+ structures. Box indicates region in (B). (B) High magnification image of (A). (C) K20+ taste bud (white arrow) with intragemmal βIII+, NFH- neurons (red arrow) and βIII+, NFH+ neurons (green arrow) were identified in epithelium. Dense βIII+, NFH− innervation (magenta arrow) and βIII+, NFH+ endings (yellow arrow) were present in the lamina propria below the taste bud. A putative end bulb of Krause resides below the taste bud (gray arrow). Box indicates region in (D). (D) High magnification image of (C). (E) Solitary chemosensory cell (cyan arrow) in close contact with an NFH+ epithelial fiber (green arrow). (F) Rare putative end bulbs of Krause (gray arrows) were found in the epiglottis. Box indicates region in (G). (G) High magnification image of (F). [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

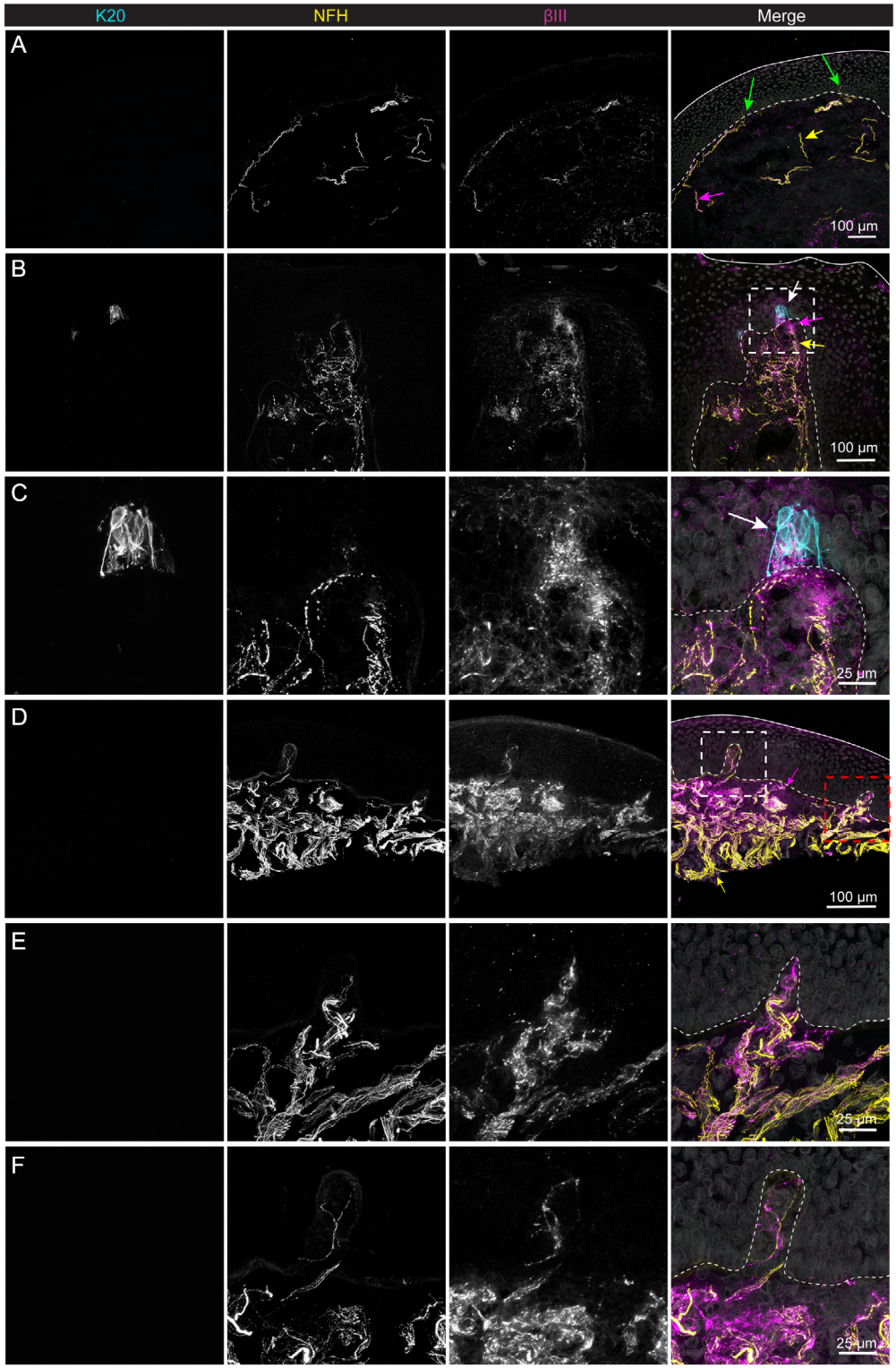

Fig. 4. Base of tongue demonstrated low innervation density of βIII and NFH in the lamina propria while the epithelium typically showed no innervation and rare K20+ structures. Solid lines indicate epithelial surface. Dashed lines indicate epithelial-lamina propria border. (A) Representative image of base of tongue tissue. Relatively sparse βIII+, NFH− (magenta arrows) and βIII+, NFH+ (yellow arrows) innervation was regularly observed in the lamina propria. Rare NFH+ nerve fibers penetrating the epithelium were found (green arrows). (B) K20+ taste buds (white arrow) were identified in 6/23 base of tongue biopsies. These tissue samples were associated with higher density of βIII+, NFH− (magenta arrow) and βIII+, NFH+ (yellow arrow) innervation within the lamina propria. Box indicates region in (C). (C) High magnification image of (B). (D) One unique base of tongue biopsy demonstrated unusually dense βIII+, NFH− (magenta arrow) and βIII+, NFH+ (yellow arrow) innervation within the lamina propria. (E) High magnification image of (D) (as outlined in red dotted box). (F) High magnification image of (D) (as outlined in white dotted box). [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

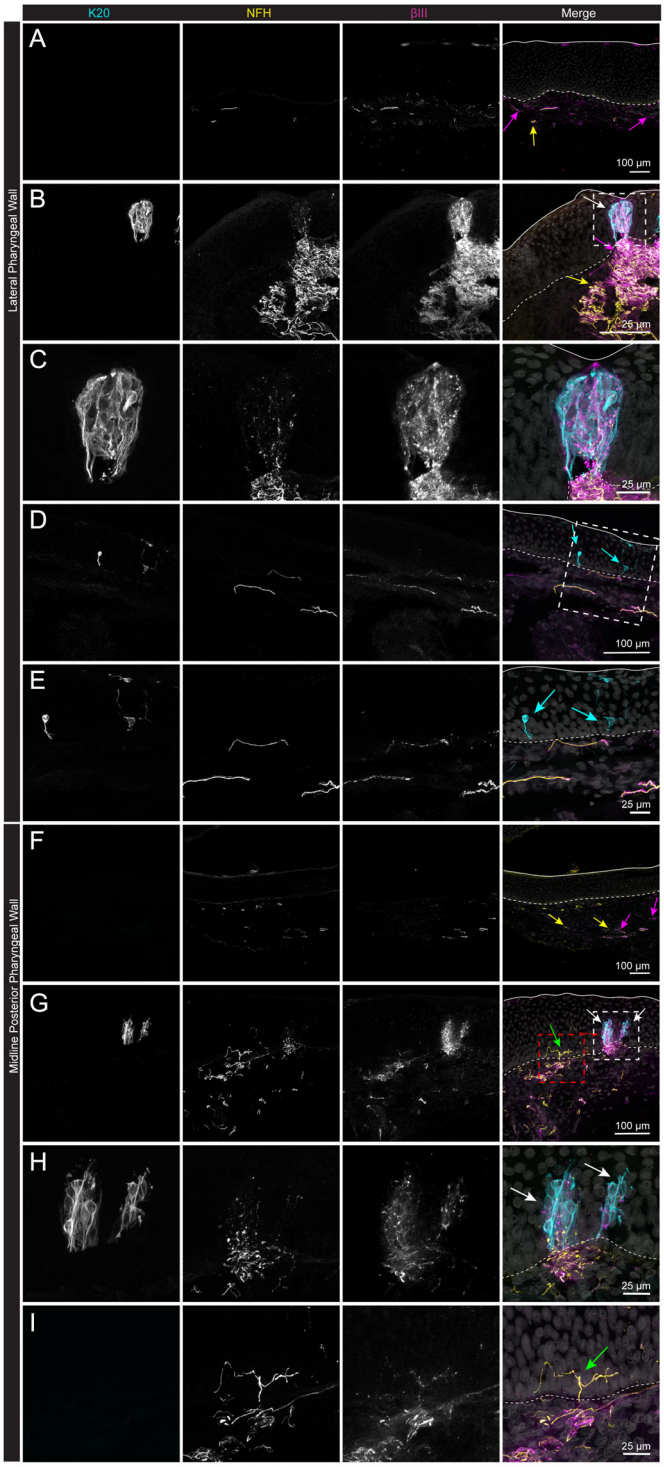

Fig. 5. Lateral and midline posterior pharyngeal wall demonstrated low innervation density of βIII and NFH, and K20+ structures were rarely observed. Solid lines indicate epithelial surface. Dashed lines indicate epithelial-lamina propria border. (A) Representative image of the lateral pharyngeal wall, showing occasional βIII+, NFH− (magenta arrows) and βIII+, NFH+ (yellow arrow) innervation density in the lamina propria. (B) Occasional K20+ taste buds were identified in lateral pharyngeal wall (white arrow, present in 3/25 biopsies). These tissue samples were associated with higher βIII+, NFH− (magenta arrow) and βIII+, NFH+ (yellow arrow) innervation density within the lamina propria. Box indicates region in (C). (C) High magnification image of (B). (D) Rare K20+ solitary chemosensory cells (cyan arrows) were observed in lateral pharyngeal wall in 3/25 biopsies. When present, solitary chemosensory cells most often occurred in proximity to other solitary chemosensory cells or taste buds. Box indicates region in (E). (E) High magnification image of (D). (F) Representative image of the midline posterior pharyngeal wall, showing minimal βIII+, NFH− (magenta arrow) and βIII+, NFH+ (yellow arrow) innervation density in lamina propria. (G) Rare taste buds were observed in midline posterior pharyngeal wall biopsies (white arrows, present in 2/24 biopsies), here seen with intragemmal βIII+, NFH− (red arrow) innervation and in close proximity to rare epithelial βIII+, NFH+ fibers (green arrow). (H) High magnification image of (G) (as outlined in white dotted box). (I) High magnification image of (G) (as outlined in red dotted box). [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

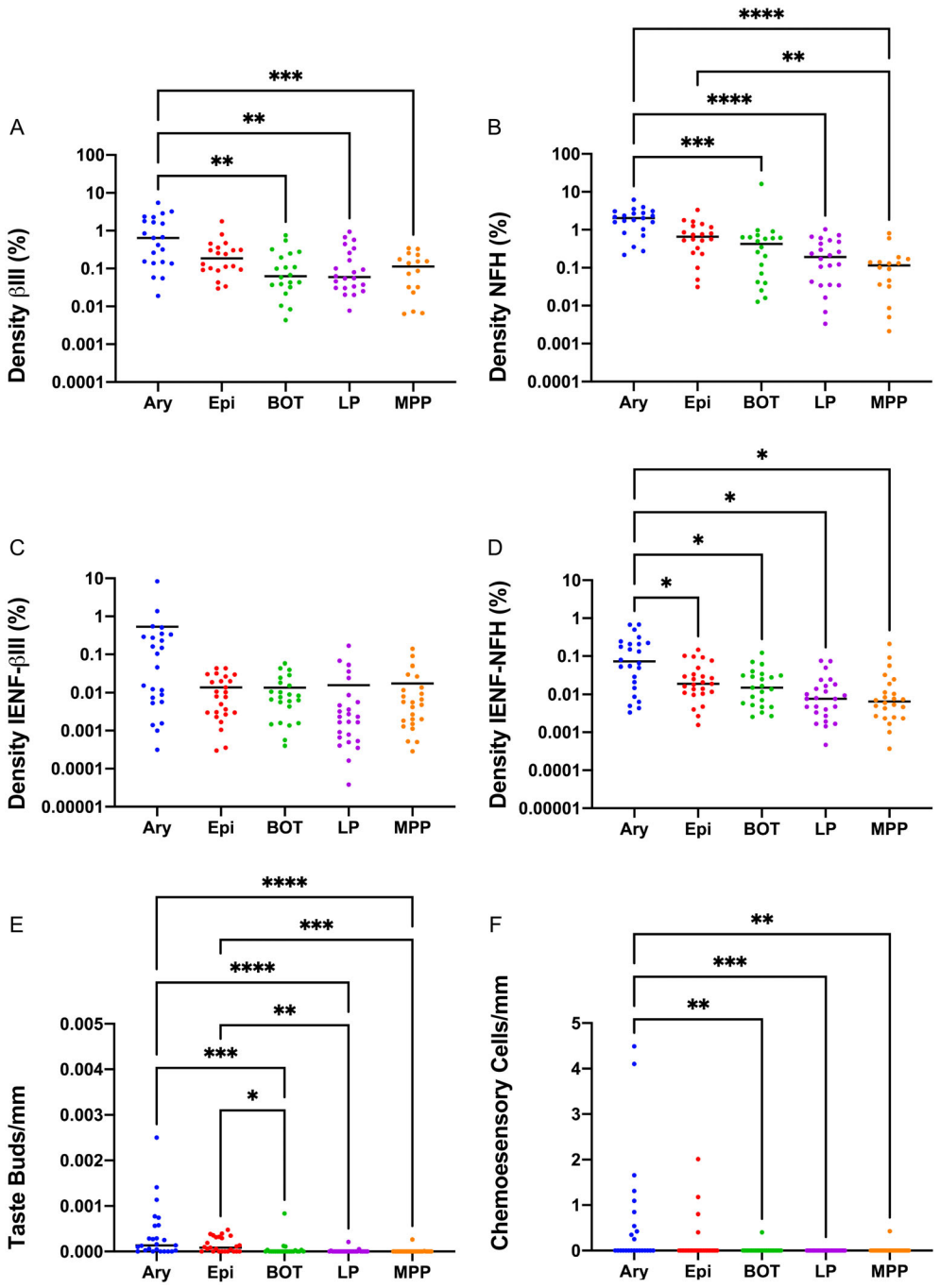

Fig. 6. Sensory cell and neuron density comparisons across tissues. Black line denotes median, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. ARY = Arytenoid; BOT = Base of tongue; EPI = Epiglottis; LP = Lateral pharynx; MPP = Midline posterior pharynx. Lamina propria measurements: N = 21 ARY, 20 EPI, 20 BOT, 22 LP, 16 MPP. Epithelial analysis: N = 24 ARY, 25 EPI, 23 BOT, 25 LP, 24 MPP. Taste bud & chemosensory cells: N = 25 ARY, 25 EPI, 23 BOT, 25 LP, 24 MPP. (A) Density of βIII within the lamina propria. Kruskal-Wallis ANOVA ($p = 0.0003$). Dunn's post-hoc analysis: Arytenoid versus BOT ($p = 0.002$), Arytenoid versus LP ($p = 0.003$), and Arytenoid versus MPP ($p = 0.006$). (B) Density of NFH within the lamina propria. Kruskal-Wallis ANOVA ($p < 0.0001$). Dunn's post-hoc analysis: Arytenoid versus BOT ($p = 0.0007$), Arytenoid versus LP ($p < 0.0001$), Arytenoid versus MPP ($p < 0.0001$), and Epiglottis versus MPP ($p = 0.007$). (C) Density of βIII within the epithelium. Brown-Forsythe analysis (non-significant). (D) Density of NFH within the epithelium Brown-Forsythe ($p < 0.0001$). Dunnett's post-hoc analysis: Arytenoid versus Epiglottis ($p = 0.045$), Arytenoid versus BOT ($p = 0.026$), Arytenoid versus LP ($p = 0.015$), and Arytenoid versus MPP ($p = 0.023$). (E) Density of Taste buds. Kruskal-Wallis ($p < 0.0001$), Dunn's post-hoc: Arytenoid versus BOT ($p = 0.0007$), Arytenoid versus LP ($p < 0.0001$), Arytenoid versus MPP ($p < 0.0001$), Epiglottis versus BOT ($p = 0.035$), Epiglottis versus LP ($p = 0.0009$), and Epiglottis versus MPP ($p = 0.0005$). (F) Density of solitary chemosensory cells. Kruskal-Wallis ($p = 0.0001$), Dunn's post-hoc: Arytenoid versus BOT ($p = 0.002$), Arytenoid versus LP ($p = 0.0003$), Arytenoid versus MPP ($p = 0.002$). [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

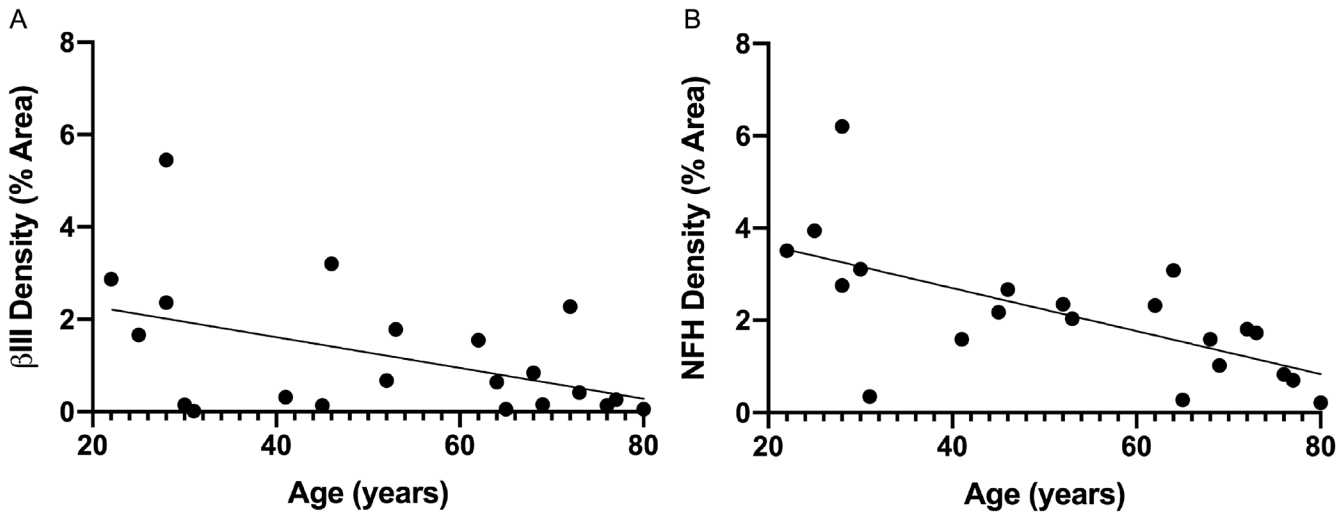

Fig. 7. Density of all neurons and NFH+ neurons in the lamina propria of the arytenoid is reduced with age. N = 21. (A) Arytenoid βIII innervation density was analyzed using simple linear regression, and showed age-related decline in the lamina propria (Slope = −0.03332, $R^2 = 0.2161$, $p = 0.0337$). (B) Arytenoid NFH innervation density was analyzed using simple linear regression, and showed age-related decline in the lamina propria (Slope = −0.04660, $R^2 = 0.4124$, $p = 0.0017$).

sites and innervation density subtypes in comparison with donor age (Figs 7, S1; Tables S2, S5–S10). Arytenoid was the only site that showed significant correlation with age, displaying a reduction in βIII and NFH density in the lamina propria. These data demonstrate that the arytenoid experiences an age-related decline in lamina propria neuronal afferent density as well as a reduction in putative mechanosensory, NFH+ peripheral neurons.

DISCUSSION

Proper sensory innervation is integral to swallowing; yet, the distribution of normal human peripheral neuronal substrates involved in swallowing and airway defense are not well understood.[1,17,19,20,41,44] This study aimed to describe and compare the density and distribution of sensory endings of laryngopharyngeal sites from healthy human donors. Of the sites examined, we found the arytenoid is the most densely innervated with both myelinated and unmyelinated sensory afferents as well as chemosensory structures. This suggests that the arytenoid is well poised to convey information about a diverse array of sensory stimuli. Furthermore, we found that innervation in the arytenoid declines with age, providing a potential sensory basis for reduction in swallowing abilities and airway defense mechanisms with aging.[11,12]

The larynx plays a vital role in airway protection, detecting various mechanical and electrical stimuli during swallowing.[58–61] Water, salt, acid, and force evoke defensive reflexes such as apnea, vocal fold adduction, pharyngeal swallow, and cough to prevent life-threatening events such as pulmonary aspiration and choking.[17,30,62–67] The sensory afferents that underlie airway protection and swallow are beginning to be understood.[17] Results from this study build on previous investigations into the organization of sensory afferents in the upper airway.[1,19] We found that epiglottis and arytenoid were densely innervated with intraepithelial nerve fibers originating from both unmyelinated and myelinated neurons, end bulbs of Krause, and abundant chemosensory structures. This suggests that these two sites are capable of transducing a rich array of chemical, thermal, and mechanosensory stimuli. How subpopulations of sensory endings contribute to upper airway functions has yet to be identified. The importance of the epiglottis and arytenoid function in protecting the airway during swallowing is consistent with the highly dense and complex sensory innervation pattern we describe.

Mechanical stimulation of the base of tongue and pharyngeal wall initiate swallow or gag reflex, suggesting the importance of mechanosensory innervation to these sites.[57,68] We found that base of tongue and both pharyngeal sites had minimal innervation in the lamina propria and epithelium and had very few chemosensory structures. The low density of sensory innervation of these sites suggests they may rely on sensory innervation from deeper or more sparse areas that were not quantified in this study or that they play a less complex role in transduction compared to laryngeal sites. Alternatively, the relatively large surface area of these structures could compensate for the low innervation density. No significant change was seen in innervation densities in these sites across age, suggesting that peripheral sensory innervation to these structures may not play a role in presbyphagia, though broader sampling of these relatively large structures would be useful in future studies.

Previous studies have shown age-related decline in the number of taste buds present in the tongue and epiglottis of human cadavers, but none to our knowledge have investigated sensory innervation of these structures across age.[69,70] This study did not identify decreased taste buds in the epiglottis across ages as in previous studies.[69] This may be due to the uneven distribution of

taste buds and the limitations of biopsied tissue from living human donors as opposed to analysis of cadaveric epiglottis in their entirety, or differences in power due to sample size (N = 25 vs. N = 237).[69] Another study found reduction in circumvallate taste bud density with age in cadavers (N = 241)[62]; similar results were not found in base of tongue, which generally had few chemosensory cells present. The arytenoid was the only site we found to have significant changes in innervation across the lifespan. Putative mechanosensory innervation density was more highly correlated with age and had a steeper negative slope than that of all neurons suggesting that, though innervation overall declines with age, myelinated, mechanosensory innervation is more impacted. This finding suggests that myelinated, putative mechanosensory neurons may play a more important role in the increasing prevalence of swallowing dysfunction with age compared with unmyelinated neurons. Future studies are needed to directly test this possibility.

Additionally, though study participants were excluded from participation based on history of neurological or muscular disease, inflammatory conditions, pain conditions, and history of chemotherapy or radiation, a detailed medical history including diagnosis of diabetes, reflux, and history of alcohol or smoking was not factored into data analysis. Future studies investigating the relationship between such factors and sensitivity of the laryngopharynx would be helpful in elucidating potential roles in dysphagia.

CONCLUSION

This report describes the distribution of mechanosensory and chemosensory innervation of human laryngopharyngeal tissue derived from healthy living donors and begins to establish the architecture of healthy adult sensory innervation. Findings demonstrate age-related decline in overall arytenoid innervation and in myelinated, putative mechanosensory innervation density, which may be a contributing factor to presbyphagia. Future studies comparing innervation densities between clinical dysphagia as well as subclinical swallowing dysfunction and innervation density in healthy controls would be beneficial in elucidating the role of somatosensory innervation in swallowing dysfunction.

ACKNOWLEDGMENTS

We thank Michael Montalbano for assistance in IRB preparation. We thank Jessica Campbell and Dr. Yin Yiu for assistance with biopsy collection. We thank Jimmy Duong for help with statistical analysis.

FUNDING INFORMATION

This work was supported by the Interdisciplinary Research Initiatives Seed (IRIS) Fund Program (EAL, MST, MJP, YM), Thompson Family Foundation (YM), and the Columbia University Vagelos College of Physicians and Surgeons Deans Research Fellowship (CLC).

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