

Original Research

## Lingual innervation in male and female marmosets

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## ABSTRACT

Several gaps in knowledge exist in our understanding of orofacial pain. Some of these include type of peripheral sensory innervation in specific tissues, differences in innervation between sexes and validation of rodent studies in higher order species. The current study addresses these gaps by validating mouse studies for sensory innervation of tongue tissue in non-human primates as well as assessing sex-specific differences. Tongue and trigeminal ganglia were collected from naïve male and female marmosets and tested for nerve fibers using specific markers by immunohistochemistry and number of fibers quantified. We also tested whether specific subgroups of nerve fibers belonged to myelinating or non-myelinating axons. We observed that similar to findings in mice, marmoset tongue was innervated with nerve filaments expressing nociceptor markers like CGRP and TRPV1 as well as non-nociceptor markers like TrkB, parvalbumin (PV) and tyrosine hydroxylase (TH). Furthermore, we found that while portion of TrkB and PV may be sensory fibers, TH-positive fibers were primarily sympathetic nerve fibers. Moreover, number of CGRP, TrkB and TH-positive nerve fibers were similar in both sexes. However, we observed a higher proportion of myelinated TRPV1 positive fibers in females than in males as well as increased number of PV + fibers in females.

Taken together, the study for the first time characterizes sensory innervation in non-human primates as well as evaluates sex-differences in innervation of tongue tissue, thereby laying the foundation for future orofacial pain research with new world smaller NHPs like the common marmoset.

## Introduction

Orofacial pain prevails in approximately 22% of the population in the United States (Gilkey and Plaza-Villegas 2017) and affects several structures of the head and neck, that can lead to chronic pain conditions of multifactorial etiology. Management of orofacial pain is challenging due to lack of sufficient pharmacotherapies as well as thorough understanding of the mechanisms and pathophysiology of orofacial conditions. To this end, knowledge of the type of peripheral sensory innervation in orofacial tissues is crucial in allowing to further our understanding of how peripheral injuries may produce pain in specific tissue types. Using rodent models, we and others have revealed that type of sensory innervation is different between the DRG and the TG systems as well as can be different between tissue types (Hockley et al. 2019; Lindquist et al. 2021; Nguyen et al. 2017; Wu et al. 2018). Moreover, recent studies with human tissues have demonstrated that there exist significant differences in genomic profiles of sensory neurons between rodents and humans (Nguyen et al. 2021; North et al. 2019; Tavares-

Ferreira et al. 2022; Yang et al. 2022), emphasizing the need to further explore sensory systems of higher order species. However, while these reports have uncovered genomic profiles of DRG and TG neurons in humans, identification of specific sensory innervation within tissue types as well as assessment of sex-specific innervation of each subtype within specific tissues, is limited with higher order species. While, employing human tissues provide an ideal means of validating rodent studies and increasing translatability, use of non-human primate (NHP) tissues provides an excellent substitute due to their close phylogenetic relationship to humans, in times when use or procurement of human specimens pose a challenge. The common marmoset (*Callithrix jacchus*) is one of the most frequently used NHPs in biomedical research as its size and weight (approx. 350–400 g) are approximately that of a rat, making it easiest to handle, breed and maintain (Tardif et al. 2011). Besides, they do not transmit diseases to human, are docile creatures and require less space and monetary cost. Moreover, because of its similarity of size with the rat, transferring protocols, procedures and models established in a rat, to the marmoset, become feasible and efficient. To this end, two

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studies have assessed pain behaviors during disease in marmosets ((Arnold et al. 2011; Vierboom et al. 2010), although no study till date have used these species to study orofacial pain.

In the current study we utilized marmosets to characterize subtypes of sensory innervation expressed in the tongue, as many chronic orofacial pain conditions such as oral mucositis, burning mouth syndrome and oral cancer, arise in this very important orofacial organ (Ghurye and McMillan 2017; Jaaskelainen and Woda 2017; Chaplin and Morton 1999; Epstein and Stewart 1993; Keefe et al. 1986; Saxena, Gnanasekaran, and Andley 1995; Cuffari et al. 2006; Grayson et al. 2022; Grayson, Furr, and Ruparel 2019; Ruparel et al. 2015; Scheff et al. 2022; Lam and Schmidt 2011; Scheff et al. 2019; Maria et al. 2016; Villa and Sonis 2015; Yang et al. 2018). We used immunohistochemistry and known molecular markers to assess sensory innervation and expression of subtypes in the tongue, compared the number of axonal subtypes between sexes and confirmed the expression of markers in TG neurons. Taken together, the study not only makes significant progress in understanding sex-dependent differences in innervation of orofacial tissues in NHP, but also establishes a segway of using marmosets to model orofacial pain in the future.

## Materials and methods

### Animals

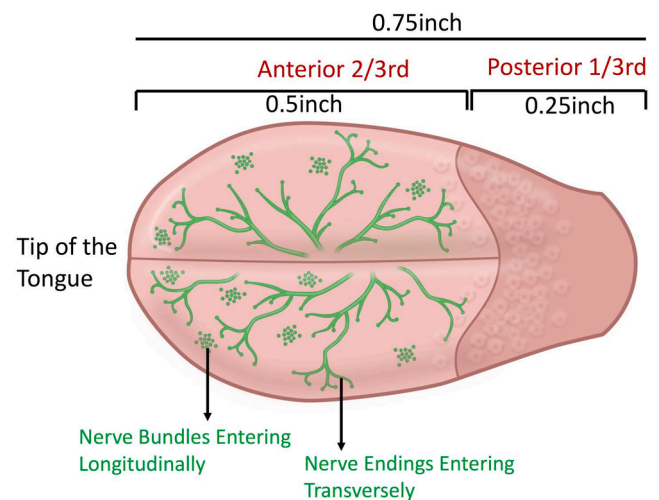
Eleven-year-old adult male and female marmosets (*Callithrix jacchus*) were used for all experiments. Naïve animals that were undergoing necropsy at the Texas Biomed Research Institute (TBRI) animal resource were used. Two animals for each sex were used for the study. All animal experiments were designed to minimize the number of animals and animal suffering, approved by the UTHSCSA and TBRI IACUC and conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

### Tissue collection and processing

Animals were sacrificed at the time of necropsy and trigeminal ganglia (TG), and tongue tissues were collected in 4% paraformaldehyde (PFA). Tissues were fixed in 4% PFA for an hour, washed in  $3 \times 15$  min in 0.1 M Phosphate Buffer (PB), immersed in 10% sucrose at 4 °C overnight and stored in 30% sucrose at -20 °C until further use.

### Immunohistochemistry

Transverse cryosections of dissected tissues were prepared using Neg-50. TG sections were cut at 25  $\mu$ m and tongue sections at 50  $\mu$ m. The marmoset set tongue is approximately 0.75 in.. Because the lingual nerve that brings in the sensory information from the trigeminal ganglia, innervates the anterior two-thirds of the tongue, we cut the tissue to 0.5 in. from the tip of the tongue to approximately section the anterior two-thirds of the tissue (Fig. 1). Immunostaining was performed as described previously (Chodroff et al. 2016; Wu et al. 2018). Briefly, sections were washed and incubated with blocking solution consisting of 4% normal donkey serum (Sigma, St. Louis, MO), 2% bovine gamma-globulin (Sigma-Aldrich, St. Louis, MO) and 0.3% Triton X-100 (Fisher Scientific) in 0.1 M PBS for 90 min at room temperature (RT). Tissue sections were then incubated overnight at RT with primary antibodies diluted in blocking solution. Details of the primary antibodies used are listed in Table 1. Sections were then rinsed and incubated with secondary antibodies in blocking solution for 90 min at RT. Alexa Fluor conjugated Donkey secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, Pennsylvania, USA). Tissue sections were then washed  $3 \times 5$  min in 0.1 M PBS, following which slides were subjected to 1:20 true black solution (Biotium, Freemont, CA, USA) (diluted in 70% ethanol) for 45 s. Slides were then washed  $2 \times 10$  min with 0.1 M PBS and two quick rinses in ddH<sub>2</sub>O, air-dried in the dark, and



**Fig. 1.** Schematic of the Area of Nerve Fiber Imaging and Quantification in Marmoset Tongue Tissue. A depiction of transverse section of marmoset tongue is shown. The tissue is about 0.75 in. in length. The lingual nerve innervates the anterior two-thirds of the tissue. Therefore, the tissue was cut and sectioned at 0.5 in. from the tip of the tongue. Sections contained a combination of nerve endings running transversely along the section as well as nerve bundles entering the tissue longitudinally. All imaging fields with nerve endings and nerve bundles in the anterior two-thirds of the tissue were included for imaging and quantification.

**Table 1**  
List of Primary Antibodies.

Marker	Company	Catalog Number
NFH	Biogen	822,601
Tyrosine Hydroxylase	Pel-freeze	P40101-150
CGRP	Synaptic Systems	414,004
TRPV1	Novus Biologicals	NBP1-71774SS
Parvalbumin	Novus Biologicals	NB120-11427SS
TRKB	R and D Systems	F397-SP11998
GFAP	Agilent Dako	Z0334
Alpha-SMA	Sigma	C6198
PGP9.5	Millipore	AB1761-I

cover-slipped with Vectashield Antifade Mounting Medium (Vectorlabs, Burlingame, CA, USA). Sections were evaluated and images obtained with Nikon C1 confocal microscope. Multiple images were acquired using fixed acquisition parameters and identical laser gain settings across all groups of each tissue type with a 20  $\times$  objective. All images obtained were z-stack images to cover the entire depth of the sections. Laser gain settings were determined such that 'no-primary control' did not show any positive staining.

### Quantification of fibers

Because only the anterior-two third of the tongue tissue was sectioned, all imaging fields containing nerve fibers and nerve bundles of the tissue section were considered for imaging and quantification (Fig. 1). All images were edited identically using identical threshold settings. To quantify the axon groups, number of nerve fibers within each image of the tongue tissues were counted manually post image editing using Adobe Photoshop 2023 software for each of the marker tested. Because all images were obtained identically with the same magnification and microscope, the area of each image was identical. The entire area of the image was considered as region of interest for every image. Each fiber was counted individually, however, fibers that were grouped together and couldn't be separated were counted as one. In addition to the network of nerve fibers that spread along the transverse

section of the tongue, we also observed nerve bundles that entered the section longitudinally and stained as 'dots' (Fig. 1). Each dot was considered as a nerve bundle and therefore counted as one. These nerve bundles were very clearly distinguished from artefact, debris or background in the images as they occurred in groups and colocalized with NFH. Fibers that were part of the fungiform papillae were not counted to exclude taste innervation. All subgroup markers were co-stained with Neurofilament Heavy (NFH) to quantify the number of fibers colocalizing with NFH and determine the percentage of specific subgroups expressed in myelinating and unmyelinating fibers. All quantification was performed by two independent blinded observers.

### Statistical analyses

GraphPad Prism 8.0 (GraphPad, La Jolla, CA) was used for statistical analysis. Data are presented as mean  $\pm$  standard error of mean (SEM). Differences between groups were assessed by Unpaired Student's t-Test or two-way ANOVA with Sidak's correction. Statistical significance was established based on a two-sided alpha of 0.05 for all tests. Sample sizes were designed to generate an 80% power at a two-sided  $P < 0.05$ .

## Results

### Differences in lingual innervation between male and female marmosets

We tested the expression of axon subgroups of lingual innervation of naïve male and female marmosets by evaluating expression of Glial Fibrillary Acidic Protein (GFAP) as a pan-nerve filament marker. GFAP was robustly expressed in lingual nerve fibers of both sexes of marmosets and in quantifying the number of fibers expressing GFAP, we found that tongue tissue of males had significantly higher number of nerve fibers compared to females (approx. 2.5 fold higher in males, Unpaired Student T-Test  $p = 0.0002$ ) (Fig. 2A). We further tested the nerve fibers for their myelination status using Neurofilament Heavy (NFH) as a marker, to assess the number of myelinating A fibers and unmyelinating C fibers. As indicated in Fig. 2B, an equal percentage of GFAP expressing fibers were found to be NFH-positive (NFH+) and NFH-negative (NFH-) in males, whereas a higher percentage GFAP+ filaments in females were NFH- compared to NFH+ (88.5% NFH- vs 11.5% NFH+) suggesting that the lower numbers of fibers in females may be attributed to reduced number of myelinating nerves. Representative images of GFAP and NFH staining are shown for both sexes (Fig. 2C and 1D). Accordingly, we plotted the number of NFH fibers counted throughout the study in the tongue of male and female marmosets and observed a significant reduction of NFH expressing fibers in females compared to males (Fig S1). These data suggests that female marmosets may have reduced innervation of A fibers in the tongue compared to males.

### Expression of nociceptors in marmoset tongue of both sexes

To test the innervation of nociceptors in NHP tongue tissues, we stained for two known markers of nociceptors: CGRP and TRPV1, in tongue sections of male and female marmosets. CGRP expressing nerve fibers were expressed in tongue of marmosets and as shown in Fig. 3A, we found no difference between the number of peptidergic fibers (i.e CGRP+ fibers) in males and females (Unpaired Student's T-test,  $p = 0.25$ ). Alongside, in estimating the percentage of CGRP fibers colocalizing with NFH, our data revealed that 30% of peptidergic fibers were myelinated A fibers and 70% were unmyelinating C fibers (Fig. 3B) and that there was no difference in these proportions between males and females (Fig. 3B, 3C and 3D). As for TRPV1 expressing fibers, we did not observe significant differences between the number of TRPV1+ fibers between males and females (Fig. 4A); however percentage of myelinating TRPV1 fibers as assessed by NFH/TRPV1 co-staining, was significantly higher in females than males (Fig. 4B, 4C and 4D). An average of 13% of all TRPV1 expressing fibers were A fibers with

corresponding 87% being C fibers in males, whereas 27% of all TRPV1 fibers were A fibers and 70% were C fibers in females (2-way ANOVA,  $p = 0.04$ ) (Fig. 4B).

### Expression of other sensory neuronal subgroups in tongue of male and female marmosets

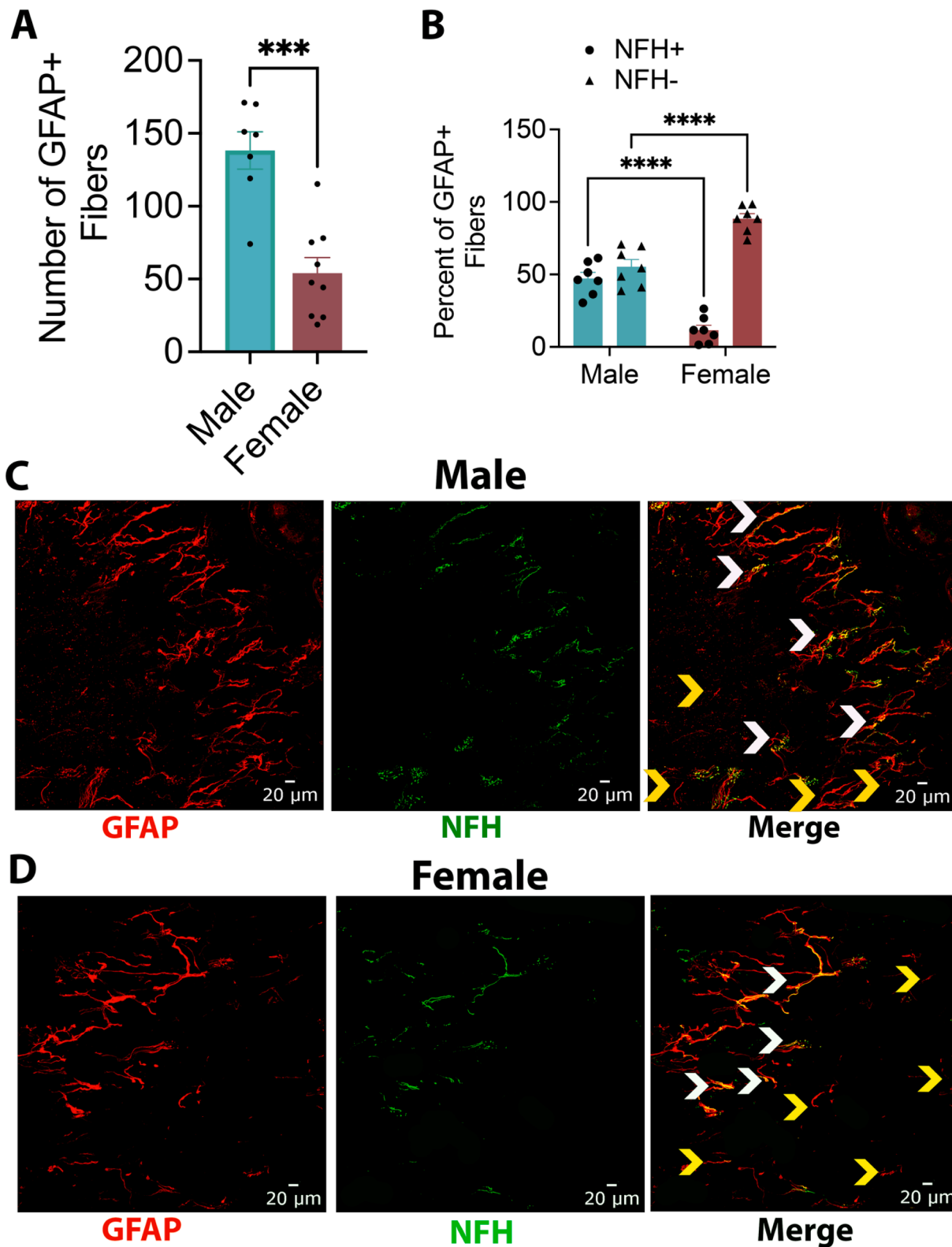
In addition to characterizing nociceptors, we also evaluated expression of additional markers that are known to be expressed in subgroups of sensory neurons (Patil et al. 2018; Usoskin et al. 2015; Wu et al. 2018)). We tested expression of TrkB and Parvalbumin (PV) in lingual nerve fibers of both marmoset sexes. Fig. 5A shows that TrkB expressing fibers were similarly expressed in marmoset tongue between males and females (Unpaired Student's T-test,  $p = 0.26$ ). Interestingly, majority of the TrkB expressing fibers were NFH- in both sexes (95% in males and 92% in females, 2-way ANOVA  $p = 0.88$ ) (Fig. 5B, 5C and 5D). PV expressing fibers were found to be significantly higher in females compared to males (Fig. 6A). Furthermore, of all PV+ fibers, female tongues showed a higher proportion of NFH+ fibers compared to males (23% in females and 8.6% in males, 2-way ANOVA,  $p = 0.039$ ) (Fig. 6B, 6C and 6D). Because TrkB can be expressed in sensory, motor and sympathetic neurons (Dale et al. 2017; Schaser et al. 2012; Zhai et al. 2011), and PV can be expressed in sensory and motor neurons (Adatia and Gehring 1971; Fitzgerald and Sachithanandan 1979; Luo et al. 2006), we tested the expression of both these markers in the neurons of the trigeminal ganglia. As shown in Fig S2A and S2B, TrkB and PV were found to be expressed in the neuronal soma of marmoset TG indicating that at least part of the TrkB and PV fibers observed in the tongue are likely to be trigeminal sensory afferents. Co-staining of these markers with CGRP demonstrated that both these markers may be expressed in peptidergic and non-peptidergic subgroups of trigeminal sensory neurons (Fig S2A and S2B).

### Expression of tyrosine hydroxylase (TH) in lingual nerve fibers of NHP

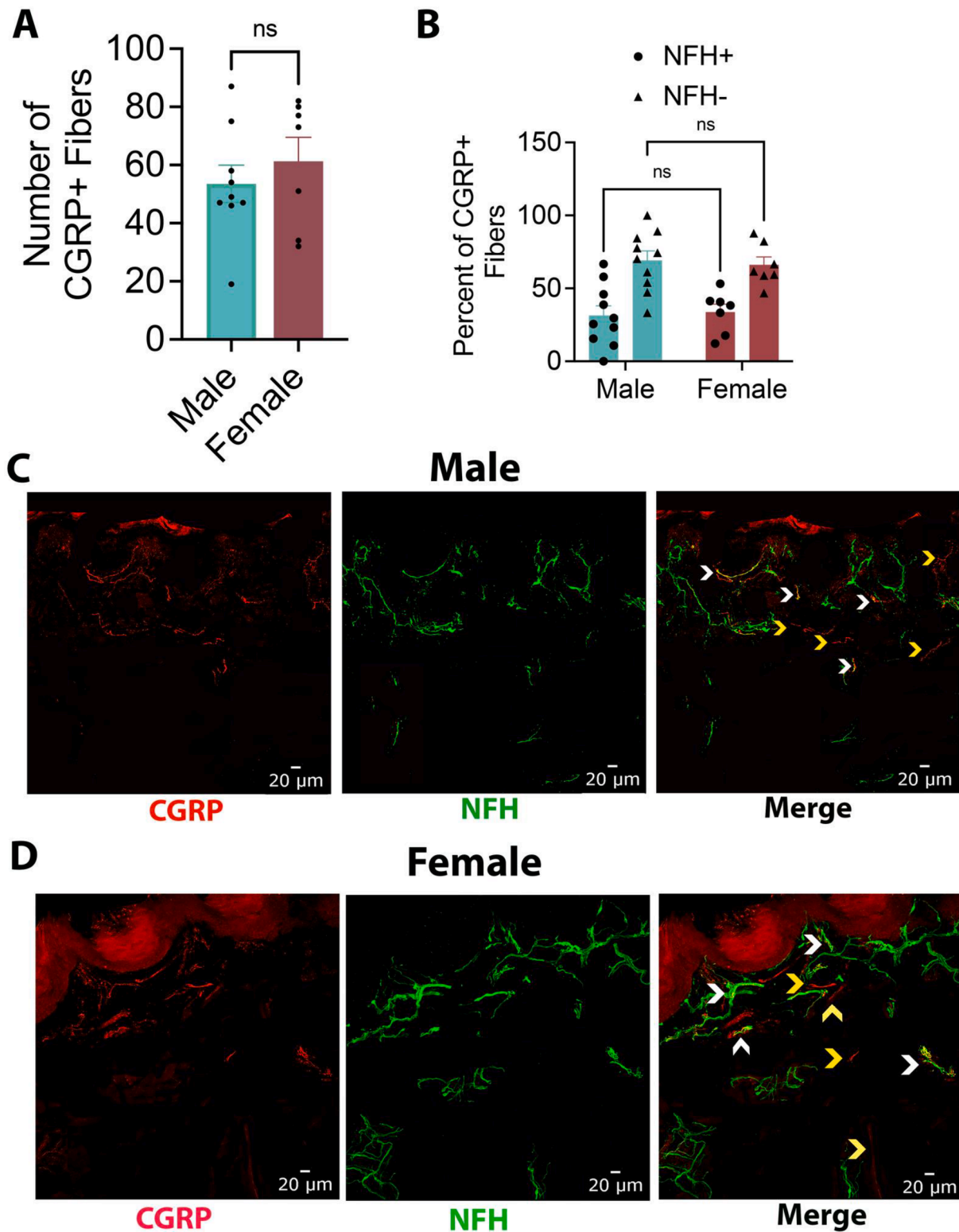
We also tested for expression of TH expressing fibers in the tongue of marmosets as TH can be expressed in sensory neurons in mice (Usoskin et al. 2015) as well as is considered a marker for sympathetic innervation that has been shown to contribute to peripheral nociception (Atherton et al. 2023; Fan et al. 2018). TH+ fibers were indeed expressed in marmoset tongue and quantification of these fibers in the tongue showed no difference in the number of TH fibers (Fig. 7A, Unpaired Student's T-test,  $p = 0.75$ ) nor in the proportion of NFH+ and NFH- TH fibers between males and females (Fig. 7B, 2-Way ANOVA,  $p = 0.99$ ). As many as 90% of TH fibers were found to be NFH- in both sexes (Fig. 7B, 7C and 7D). Furthermore, we observed very few sensory neurons in the TG tissue expressing TH (Fig S3A) as well as observed TH+ fibers run along blood vessels (stained by  $\alpha$ SMA) within the tongue tissue (Fig S3B). These data indicate that TH+ fibers in the tongue of marmosets are primarily sympathetic fibers.

## Discussion

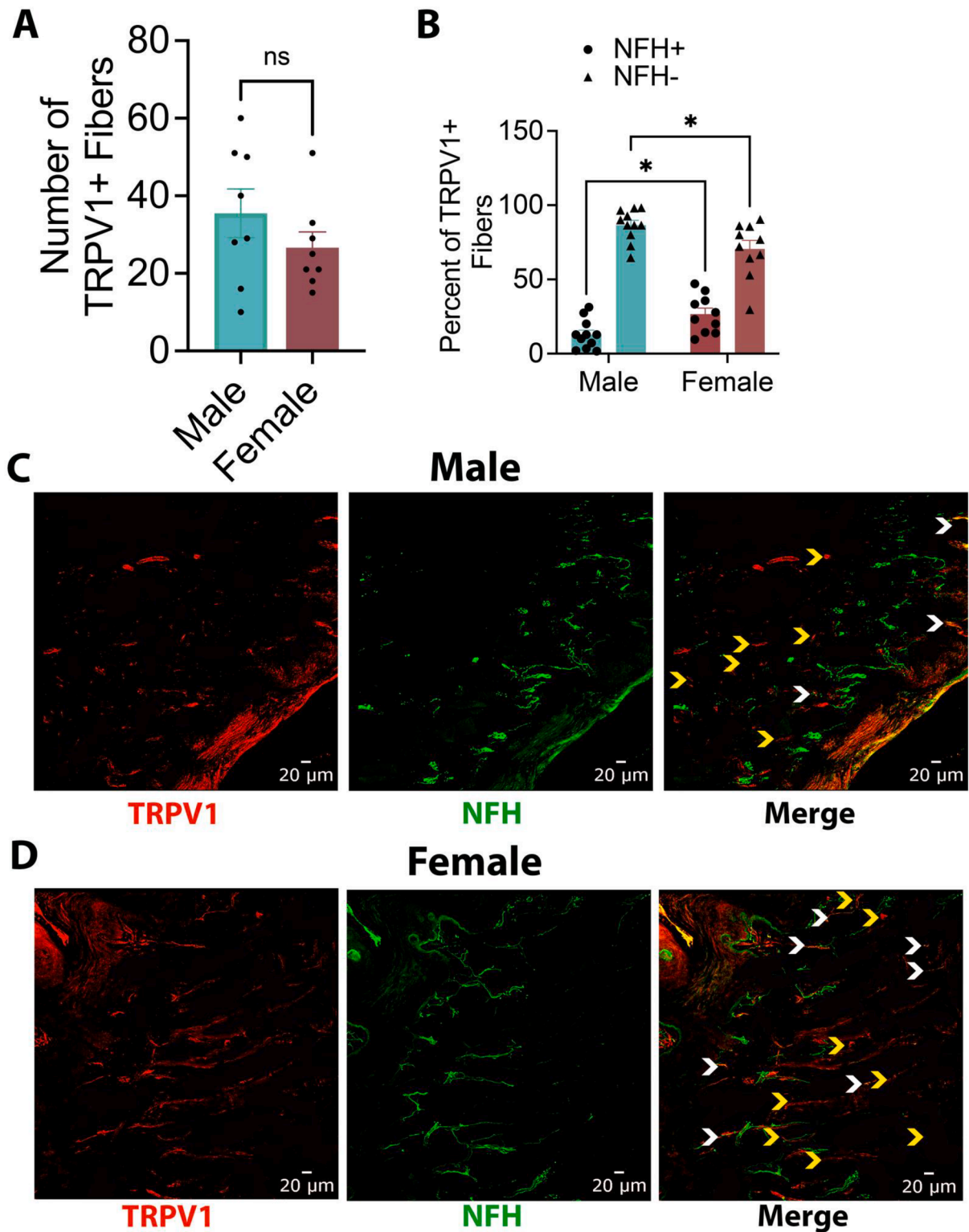
Tongue pain can arise from a range of acute and chronic orofacial conditions including oral cankers, oral thrush, glossitis, burning mouth syndrome, tongue neuralgia, oral mucositis and oral cancer (Abe et al. 2007; Menni, Boccardi, and Crosti 2004; Marable et al. 2016; Grinspan et al. 1995; Chaplin and Morton 1999; Epstein and Stewart 1993; Keefe et al. 1986). Despite this, our understanding of how tongue pain occurs is very limited. One of the hallmark features of the unique manifestations of pain symptoms, intensity and duration within each tissue type in the body, is the differences in type of peripheral innervation expressed by specific tissues. Accordingly, we have shown that tongue-innervating sensory neurons possess distinct subgroups of sensory neurons in mice (Wu et al. 2018). However, several reports have indicated that sensory neurons in mice may significantly differ from that of humans (Nguyen



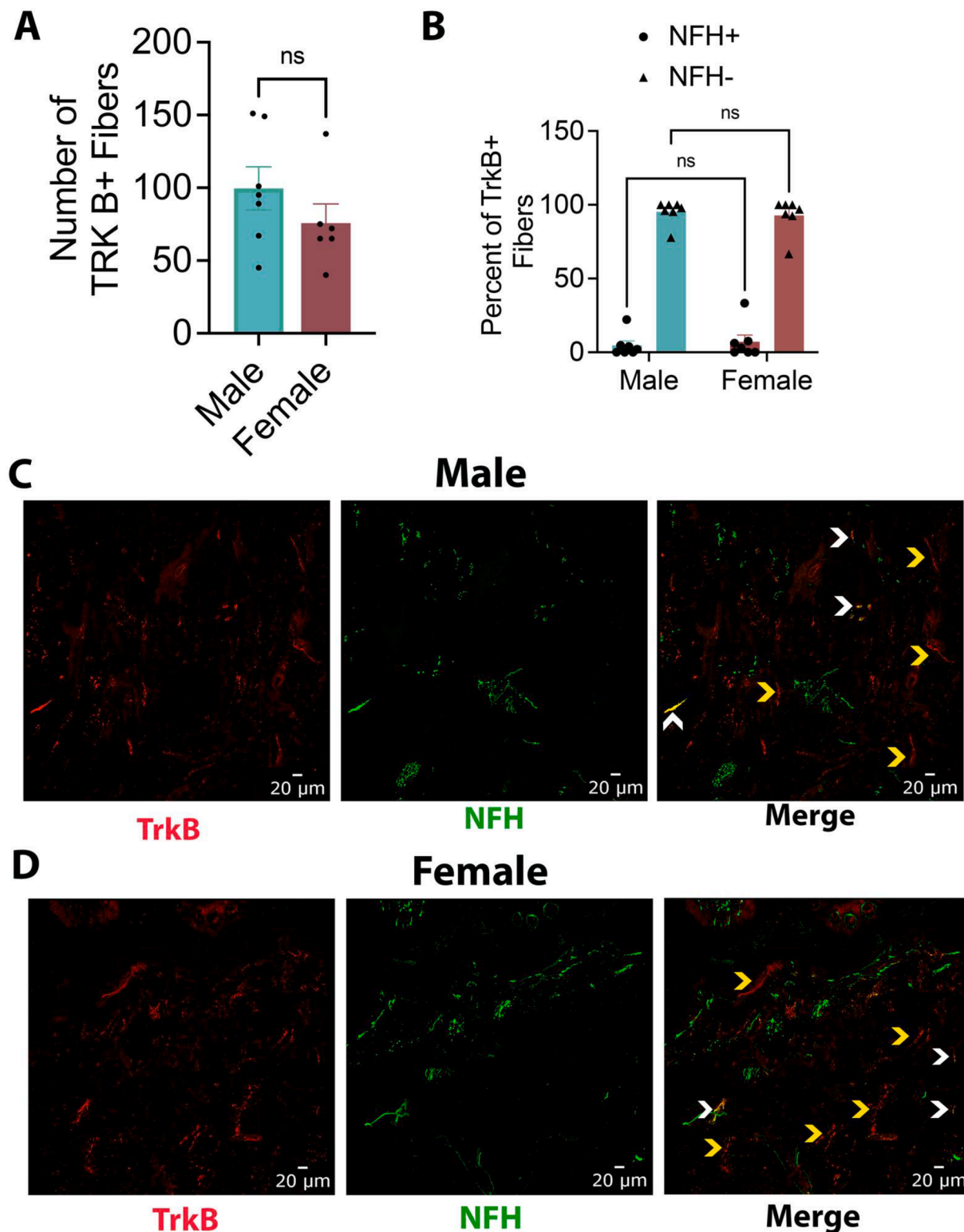
**Fig. 2.** Lingual Innervation of Male and Female Marmosets. Tongue tissues of male and female marmosets were harvested at necropsy and subjected to immunohistochemical staining of GFAP and NFH. Images were taken on a C1 confocal microscope at 20x magnification and nerve fibers and bundles manually counted. **A.** Number of GFAP + nerve fibers counted in tongue sections of male and female animals. Data are presented as mean ± SEM and analyzed by Unpaired Student T-Test at  $p < 0.05$ . **B.** Percent of GFAP + fibers expressing NFH in males and females is plotted. Data are presented as mean ± SEM and analyzed by 2-way ANOVA with Sidak post hoc test at  $p < 0.05$ . **C.** Representative image of GFAP and NFH staining in tongue of male marmosets. **D.** Representative image of GFAP and NFH staining in tongue of female marmosets. White Arrows indicate GFAP+/NFH + fibers and yellow arrows indicate GFAP+/NFH- fibers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



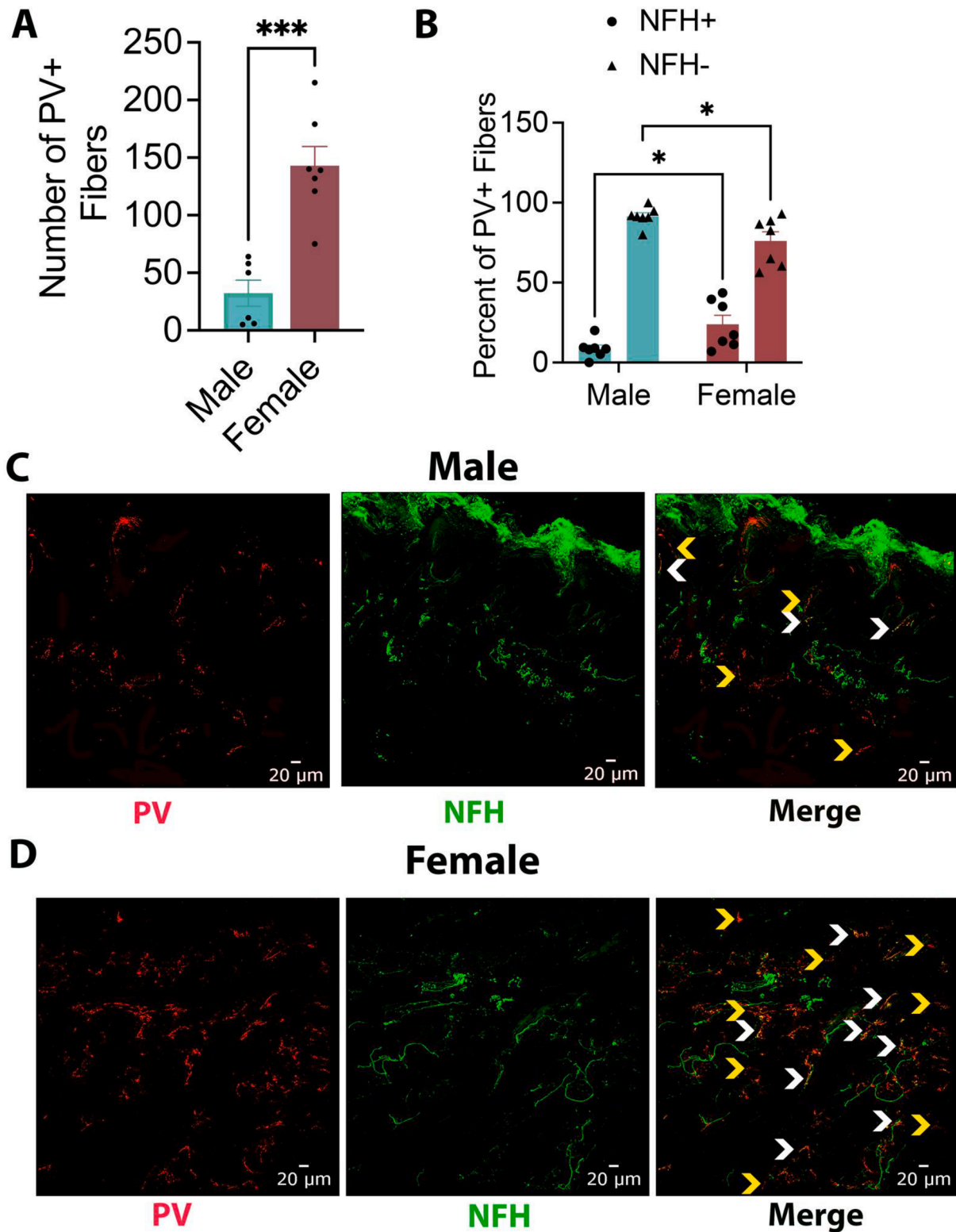
**Fig. 3.** CGRP expression in nerve fibers of Male and Female Marmosets. Tongue tissues of male and female marmosets were harvested at necropsy and subjected to immunohistochemical staining of CGRP and NFH. Images were taken on a C1 confocal microscope at 20x magnification and nerve fibers and bundles manually counted. **A.** Number of CGRP + nerve fibers counted in tongue sections of male and female animals. Data are presented as mean ± SEM and analyzed by Unpaired Student T-Test at  $p < 0.05$ . **B.** Percent of CGRP + fibers expressing NFH in males and females is plotted. Data are presented as mean ± SEM and analyzed by 2-way ANOVA with Sidak post hoc test at  $p < 0.05$ . **C.** Representative image of CGRP and NFH staining in tongue of male marmosets. **D.** Representative image of CGRP and NFH staining in tongue of female marmosets. White Arrows indicate CGRP+/NFH + fibers and yellow arrows indicate CGRP+/NFH- fibers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** TRPV1 expression in nerve fibers of Male and Female Marmosets. Tongue tissues of male and female marmosets were harvested at necropsy and subjected to immunohistochemical staining of TRPV1 and NFH. Images were taken on a C1 confocal microscope at 20x magnification and nerve fibers and bundles manually counted. **A.** Number of TRPV1 + nerve fibers counted in tongue sections of male and female animals. Data are presented as mean ± SEM and analyzed by Unpaired Student T-Test at  $p < 0.05$ . **B.** Percent of TRPV1 + fibers expressing NFH in males and females is plotted. Data are presented as mean ± SEM and analyzed by 2-way ANOVA with Sidak post hoc test at  $p < 0.05$ . **C.** Representative image of TRPV1 and NFH staining in tongue of male marmosets. **D.** Representative image of TRPV1 and NFH staining in tongue of female marmosets. White Arrows indicate TRPV1+/NFH+ fibers and yellow arrows indicate TRPV1+/NFH- fibers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

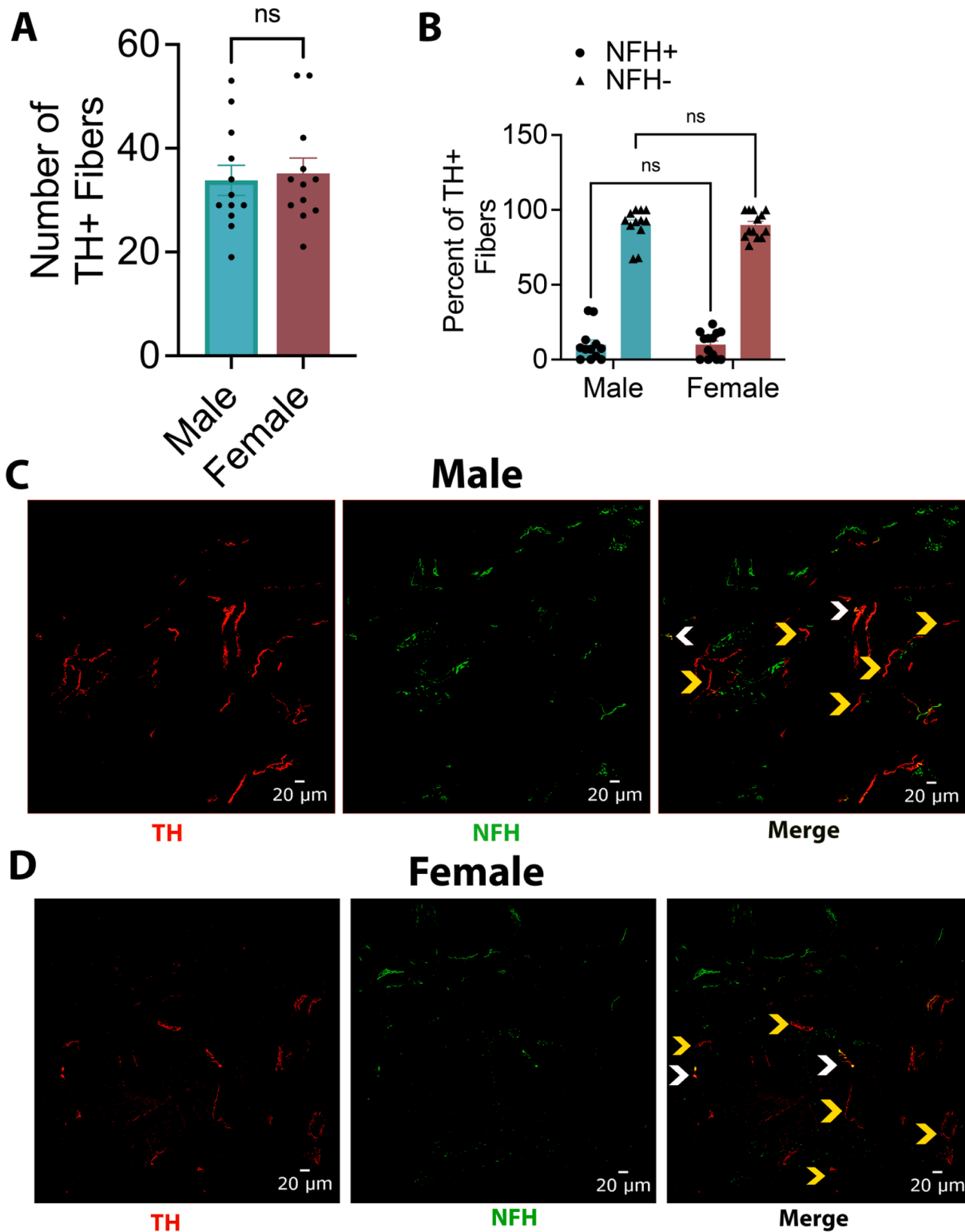


**Fig. 5.** TrkB expression in nerve fibers of Male and Female Marmosets. Tongue tissues of male and female marmosets were harvested at necropsy and subjected to immunohistochemical staining of TrkB and NFH. Images were taken on a C1 confocal microscope at 20x magnification and nerve fibers and bundles manually counted. **A.** Number of TrkB + nerve fibers counted in tongue sections of male and female animals. Data are presented as mean ± SEM and analyzed by Unpaired Student T-Test at  $p < 0.05$ . **B.** Percent of TrkB + fibers expressing NFH in males and females is plotted. Data are presented as mean ± SEM and analyzed by 2-way ANOVA with Sidak post hoc test at  $p < 0.05$ . **C.** Representative image of TrkB and NFH staining in tongue of male marmosets. **D.** Representative image of TrkB and NFH staining in tongue of female marmosets. White Arrows indicate TrkB+/NFH+ fibers and yellow arrows indicate TrkB+/NFH- fibers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 6.** PV expression in nerve fibers of Male and Female Marmosets. Tongue tissues of male and female marmosets were harvested at necropsy and subjected to immunohistochemical staining of PV and NFH. Images were taken on a C1 confocal microscope at 20x magnification and nerve fibers and bundles manually counted. A. Number of PV + nerve fibers counted in tongue sections of male and female animals. Data are presented as mean ± SEM and analyzed by Unpaired Student T-Test at  $p < 0.05$ . B. Percent of PV + fibers expressing NFH in males and females is plotted. Data are presented as mean ± SEM and analyzed by 2-way ANOVA with Sidak post hoc test at  $p < 0.05$ . C. Representative image of PV and NFH staining in tongue of male marmosets. D. Representative image of PV and NFH staining in tongue of female marmosets. White Arrows indicate PV+/NFH + fibers and yellow arrows indicate PV+/NFH- fibers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)





**Fig. 7.** TH expression in nerve fibers of Male and Female Marmosets: Tongue tissues of male and female marmosets were harvested at necropsy and subjected to immunohistochemical staining of TH and NFH. Images were taken on a C1 confocal microscope at 20x magnification and nerve fibers and bundles manually counted. A. Number of TH + nerve fibers counted in tongue sections of male and female animals. Data are presented as mean ± SEM and analyzed by Unpaired Student T-Test at  $p < 0.05$ . B. Percent of TH + fibers expressing NFH in males and females is plotted. Data are presented as mean ± SEM and analyzed by 2-way ANOVA with Sidak post hoc test at  $p < 0.05$ . C. Representative image of TH and NFH staining in tongue of male marmosets. D. Representative image of TH and NFH staining in tongue of female marmosets. White Arrows indicate TH+/NFH+ fibers and yellow arrows indicate TH+/NFH- fibers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al. 2021; North et al. 2019; Tavares-Ferreira et al. 2022; Yang et al. 2022). Thus, to validate translatability of our findings in rodents, it is crucial to assess the type of innervation in the tongue tissue of higher order species. Non-human primates (NHPs) have been long considered a very viable option for biomedical research due to their genetic and physiological similarities to humans. Their evolutionary trajectories make them particularly useful for sensory and neurological research (Austad 1997; Tardif et al. 2011). Indeed, several groups have used NHPs for pain research, however, majority of the studies have employed the old-world larger primates (Klein et al. 2021; Paterson and Turner 2022; Vardigan et al. 2018). While the larger primates are phylogenetically closest to humans, they present with several drawbacks including challenges in acquisition, maintenance, longevity and costs. On the other hand, the new world smaller NHPs (e.g marmosets, squirrel monkeys etc), due to their size, weight, lifespan and price, are a good compromise between practicality and evolutionary relationship to humans. Besides a few studies that have utilized squirrel monkeys (Paterson and Turner 2022), the smaller NHPs have not been explored in pain research. The current study for the first time explores the peripheral sensory system of the orofacial region in the common marmoset. Moreover, sex-dependent differences in tissue-specific sensory innervation have not been reported in higher order species till date. Taken together, using immunohistochemistry, we assessed the type and number of sensory innervation in tongue tissues of male and female marmosets.

We employed the traditional approach of using conventional IHC of tissue sections versus quantifying *retro*-labeled TG neurons or using whole tissue 3D imaging for the following reasons: Retro-labeling of TG neurons precludes maximizing the use of the non-human primates (NHP) that are already limited in availability and are expensive to purchase. With our approach, because there were no prior procedures performed in the animals, these animals were used to collect various tissues at necropsy for several different studies by multiple research groups and allowed us to conduct the study without incurring significant costs for purchasing the entire animal. Whole-tissue imaging has its own limitation which is that it will require a lot of animals to be able to test multiple different sensory subgroups and markers. This in turn take up significant resources. These limitations are mitigated with the use of tissue sections as the study can be conducted with considerably fewer animals and several markers can be tested from the same tissue. Given that all tissue processing, imaging and analyses were conducted the exact same way across all animals used in the study, data obtained from our work, for the first time provides information on potential similarities and differences in peripheral sensory innervation between the sexes in higher order species, that would have otherwise taken a lot more time, effort and resources. Furthermore, as stated above, the study lays the foundation for future orofacial pain research with new world smaller NHPs like the common marmoset.

Quantification of IHC images were conducted using manual counting of the number of fibers in each image throughout the study. We note that quantification can be performed either by manual quantification (Galosi et al. 2018; LoCoco et al. 2017) or by automated quantification using softwares like ImageJ (Alhilou et al. 2021a,b; Pemberton, Mersman, and Xu 2018; Schindelin et al. 2012); and analyses can be conducted for either the number of fibers (Galosi et al. 2018; LoCoco et al. 2017), length of fibers (Pemberton, Mersman, and Xu 2018; Schindelin et al. 2012) or total density of fibers within an image (Alhilou et al., 2021a,b). Automated methods have limitations and are somewhat subjective as they rely on parameters set by the experimenter, for image editing and analyses. However, it is accepted that these provide useful information as long as all images are processed and analyzed the exact same way. We chose the manual option for the following three reasons: a) It provided for greater control over the process especially when identifying nerve fibers from artifact or debris; b) we could confirm our results by two blinded observers to reduce bias and subjectivity and c) for the current study, automated methods using same set parameters for image editing

and processing, did not result in expected outcome for every single image. Specifically, the software did not include all nerve fibers within every image nor did it include the nerve bundles entering longitudinally during quantification. We chose to quantify our data as number of fibers instead of measuring length of fibers or total density of fibers within an image for two reasons: a) the length of fibers or the overall density of fibers within an image can vary from section to section due to unequal distribution of nerve fibers throughout the tissue and therefore would require way more sections from an expensive animal to obtain accurate representation of innervation density. In contrast, our approach of quantifying the number of fibers did not induce high variability as evident from all of our data and b) because of the presence of nerve bundles longitudinally entering into the section (stained as dots) (Fig. 1), our data would be skewed if quantification was performed for measuring length of fibers or total density of fibers in an image. Therefore, in our opinion, counting the number of fibers was the best method to represent specific innervation in tongue tissue sections of expensive marmosets. Further, we acknowledge that counting inseparable fibers or nerve bundles as one is not ideal; however, despite this drawback, our data demonstrates that we were able to observe similarities and differences in innervation between the sexes with reduced variability, indicating the viability of this approach.

We first evaluated for pan-innervation in the anterior two-third of the tongue tissues and our data showed that. We used GFAP as a pan-nerve filament marker as it is known to stain myelinating and non-myelinating Schwann cells (Bianchini et al. 1992; McKenzie et al. 2006; Yang and Wang 2015). However, PGP9.5 which is a widely used pan-neuronal marker (Boyd et al. 2021; Lindquist et al. 2021; Brady et al. 2004; Chou et al. 2001; Atherton et al. 2023; Agelopoulos et al. 2023), also stained well and can also be used as an alternate, for marmoset tongue tissues (Fig S4). We did not find any evidence of pan-nerve fiber staining of human tongue tissues, although we speculate that both GFAP and PGP9.5 can potentially be used as pan-nerve markers in human tongue as well. We observed robust expression of GFAP in marmoset tongue tissues of both sexes; however, females had decreased number of total GFAP + nerve fibers in the tongue compared to males (Fig. 2A) Interestingly, while myelinating and non-myelinating nerve fibers in males were found to be in equal proportion, majority of GFAP + nerve fibers in females were found to be non-myelinating. This was in accordance to the significant decrease in total number of NFH + fibers found in females. Collectively, these data suggests that the reduction in tongue nerves in females is likely due to the decreased proportion of myelinating fibers. This is the very first evidence reporting differences in myelinating nerves in tongue tissues between sexes. Whether this effect is due to differences in arborization of axons or due to the total number of neuronal cell bodies innervating the tongue is not known yet. Further, whether this effect is specific to the marmosets or translates to human or mouse tongue tissues is to be determined. The tongue is innervated with four types of nerve fibers in the anterior 2/3rd of the tissue. These include the sensory fibers, sympathetic fibers, taste receptors and motor nerve fibers. While the taste receptors are mainly present in the fungiform papillae which weren't included in our analyses, our data with the GFAP staining likely encompasses the remaining three types of innervation.

Therefore, to further evaluate sensory innervation in the tongue, we tested expression of CGRP and TRPV1 as the two markers specific to the sensory nervous system. Expectedly, CGRP and TRPV1 positive fibers were expressed in the tongue of both sexes. No difference in the number of CGRP + fibers was observed between males and females (Fig. 3A) which correlated with what has been implicated in mice (Stucky et al. 2011). The proportion of CGRP + fibers in C and A fibers was also similar between sexes (Fig. 3B). Notably, these proportions were in accordance with what we have observed in mouse tongue where 30% of CGRP positive lingual fibers in this study was found to be NFH + and similar percentage of CGRP + tongue-innervating neurons were found to be NFH + in mice (Wu et al. 2018). Similar to CGRP, numbers of TRPV1

+ fibers obtained from male and female tongue tissues were insignificant from each other in the marmoset (Fig. 4A). Scheff *et al* recently reported that oral aversion test to capsaicin produced no significant difference in sensitivity in naïve male and female mice (Scheff *et al.* 2022) indicating that mice also likely possess similar TRPV1 + lingual fibers in both sexes. In addition, it has also been reported that the numbers of both CGRP and TRPV1 positive tongue-innervating neurons are increased during tongue cancer in mice and this too occurs in both sexes similarly (Horan *et al.* 2022). These data indicate that regulation of TRPV1 expression in lingual sensory neurons during disease may not be sex-specific, although this remains to be confirmed in NHPs and humans. It is noteworthy though that while expression of TRPV1 appear to be conserved in tongue tissues within sexes, activity of TRPV1 and its mechanistic role in pain has been demonstrated to have a sex-specific role in many rodent pain models (Ducieux *et al.* 2020; Luo *et al.* 2021; Payrits *et al.* 2017) and even though these studies are conducted outside of the orofacial region, this possibility cannot be ruled out for lingual neurons during injured conditions. More intriguingly, we found that proportion of NFH + TRPV1 fibers in female tongue were significantly higher than in males (Fig. 4B) implying increased expression of TRPV1 in A fibers than C fibers in females. This is a novel finding and can have important implications in understanding the differences in manifestations of pain in females than males. For example, the type of pain experienced by females during disease may be different than that experienced by males. Scheff *et al* conveyed that in oral cancer (Scheff *et al.* 2018), female patients report significantly higher pain score for sharp pain than males. This aligns with our finding of increased TRPV1 expression in A fibers of females that could contribute to this sex-specific symptom in oral cancer; in turn pointing to a sex-specific regulatory mechanism of A-fiber nociceptors in oral cancer. It will be important to assess whether this sex-specific difference in TRPV1 expression in C and A fibers exists in mice, as majority of our understanding of sexually-dimorphic mechanisms in pain research is achieved from mouse studies.

In addition to nociceptor markers, we also tested three other markers of sensory neurons that were identified to be specific to non-nociceptor subset of sensory neurons in mouse DRGs. These included TrkB, PV and TH. TrkB in DRG neurons is known to be specifically expressed in A-low threshold mechanoreceptors (Usoskin *et al.* 2015; Li *et al.* 2011) whereas PV is known to specifically mark proprioceptors (Medici and Shortland 2015) and TH in C-low threshold mechanoreceptors (Li *et al.* 2011). We have shown expression of all three molecules in tongue-innervating TG neurons in mice and similarly, the current study shows expression of all three markers in marmoset tongue tissue.

Numbers of total TrkB expressing fibers were no different between males and females in our study. TrkB has been shown to be expressed in motor neurons including that of the tongue (Dale *et al.* 2017; Schaser *et al.* 2012; Zhai *et al.* 2011) as well as in the sympathetic nervous system (Kasemeier-Kulesa *et al.* 2015; Schober *et al.* 1998). However, expression of TrkB in marmoset TG neuronal cell soma (Fig S2A), indicates that part of the TrkB expressing fibers in the tongue are of sensory origin. Interestingly though, majority of the TrkB expressing fibers were found to be NFH- (Fig. 5B) which is in contrast to previous mouse DRG studies that report TrkB expression in A-neurons (Usoskin *et al.* 2015; Li *et al.* 2011). Notably though, we observed expression of TrkB in a wide range of lingual mouse neurons including myelinating and non-myelinating neurons as well as peptidergic and non-peptidergic neurons and did not belong to a specific subtype like in the DRGs (Wu *et al.* 2018). In further exploring the expression of two isoforms of TrkB in mouse lingual neurons, we reported that the full-length TrkB isoform is indeed primarily expressed in myelinating lingual neurons in mice and is only expressed in only 10% of all lingual neurons, but its truncated isoform that lacks the kinase domain, is expressed in wider proportion of neurons (35% of all lingual neurons) with almost equal expression of this isoform in myelinating as well as unmyelinating neurons (Grayson *et al.* 2022). Moreover, we showed that the expression of truncated TrkB in mouse lingual neurons belonged to the peptidergic as well as non-

peptidergic subgroups (Grayson *et al.* 2022) that aligns with our marmoset TG data showing TrkB expression in CGRP + and CGRP-neurons. Therefore, it wouldn't be surprising if the marmoset TG neurons also express the truncated TrkB as the predominant isoform, similar to tongue-innervating neurons in mice. Despite the predominant expression of truncated TrkB in lingual sensory neurons, the neuronal role of this isoform is yet to be explored.

In testing for PV expression in nerve fibers of the tongue, we observed that the number of PV + fibers were significantly higher in females compared to males (Fig. 6A). Correspondingly, the proportion of PV + fibers expressing NFH was also higher in females than males (Fig. 6B). This female-specific increase in PV + neurons have only been reported in one other study in the caudate putamen in the forebrain of rats (Ravenelle *et al.* 2014). PV being a marker for proprioceptors; we speculate that the primary source of these nerves may be the neurons of the hypoglossal nerve as reported previously (Adatia and Gehring 1971; Fitzgerald and Sachithanandan 1979; Luo *et al.* 2006). However, with our mouse studies we have shown that at least 12–14% of PV + sensory neurons from the trigeminal ganglia innervate mouse tongue and accordingly, we observed PV + neurons in marmoset TG neurons as well (Fig S2B). Therefore, part of the PV + fibers observed in marmoset tongue may be of sensory origin. Whether or not the increase in PV + nerves in females is contributed by the trigeminal neurons is still unknown. Since its believed that trigeminal neurons do not participate in proprioception, the role of PV + trigeminal sensory neurons in the tongue may have an entirely novel function that is yet to be investigated.

Unlike TrkB and PV, TH + nerve filaments were present in the tongue (Fig. 7); however expression of TH was found to be in very few TG neurons indicating that TH + nerves in the tongue are not sensory fibers (Fig S3A). This is in accordance to what we and others have observed in mice (Atherton *et al.* 2023; Wu *et al.* 2018). Instead, our data confirmed that TH + nerve filaments were surrounding blood vessels indicative of sympathetic innervation (Fig S3B). Sympathetic Innervation in peripheral tissues results in a crucial cross-talk with the peripheral sensory nervous system to contribute to pain during disease. Among several pain models, this cross-talk has indeed been shown to exist in the tongue and contribute to tongue cancer pain (Atherton *et al.* 2023). Sex-dependent differences in sympathetic innervation has been reported previously (Kim *et al.* 2016); however our data showed no differences between TH + lingual fibers between males and females.

Collectively, our data validates our mouse study for the expression of specific sensory nerve subtypes in marmoset tongue tissue; adds interesting insights about sex differences in tongue innervation including the nociceptors, as well as suggests that use of marmosets can potentially be combined with mouse models to improve translatability of orofacial pain research in the future.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Author Contributions

M.T performed all tongue and TG dissections, conducted immunohistochemical staining as well as analyzed the data. T.I and A.H

processed and sectioned tissues for immunohistochemistry. T.I. also performed some IHC staining. S.R. acquired all images. A.A and S.R. conceptualized and designed all experiments. S.R wrote the manuscript. All authors edited the manuscript.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ynpai.2023.100134>.

## References

- Abe, M., Sogabe, Y., Syuto, T., Ishibuchi, H., Yokoyama, Y., Ishikawa, O., 2007. Successful treatment with cyclosporin administration for persistent benign migratory glossitis. *J Dermatol* 34, 340–343.
- Adatia, A.K., Gehring, E.N., 1971. Proprioceptive innervation of the tongue. *Journal of Anatomy* 110, 215–220.
- Agelopoulos, K., Renkhold, L., Wiegmann, H., Dugas, M., Suer, A., Zeidler, C., Schmelz, M., Pereira, M.P., Stander, S., 2023. Transcriptomic, Epigenomic, and Neuroanatomic Signatures Differ in Chronic Prurigo, Atopic Dermatitis, and Brachioradial Pruritus. *J Invest Dermatol* 143, 264–272 e3.
- Alhilou, A.M., Shimada, A., Svensson, C.I., Ernberg, M., Cairns, B.E., Christidis, N., 2021a. Density of nerve fibres and expression of substance P, NR2B-receptors and nerve growth factor in healthy human masseter muscle: An immunohistochemical study. *J Oral Rehabil* 48 (1), 35–44.
- Alhilou, A.M., Shimada, A., Svensson, C.I., Svensson, P., Ernberg, M., Cairns, B.E., Christidis, N., 2021b. Nerve growth factor and glutamate increase the density and expression of substance P-containing nerve fibers in healthy human masseter muscles. *Sci Rep* 11, 15673.
- Arnold, C., Lamp, J., Lamp, O., Einspanier, A., 2011. Behavioral tests as indicator for pain and distress in a primate endometriosis model. *J Med Primatol* 40, 317–326.
- Atherton, M.A., Park, S., Horan, N.L., Nicholson, S., Dolan, J.C., Schmidt, B.L., Scheff, N.N., 2023. Sympathetic modulation of tumor necrosis factor alpha-induced nociception in the presence of oral squamous cell carcinoma. *Pain* 164 (1), 27–42.
- Austad, S.N., 1997. Small Nonhuman Primates as Potential Models of Human Aging. *ILAR J* 38, 142–217.
- Bianchini, D., De Martini, I., Cadoni, A., Zicca, A., Tabaton, M., Schenone, A., Anfosso, S., Akkad Wattar, A.S., Zaccaro, D., Mancardi, G.L., 1992. GFAP expression of human Schwann cells in tissue culture. *Brain Res* 570, 209–217.
- Boyd, J.T., LoCoco, P.M., Furr, A.R., Bendele, M.R., Tram, M., Li, Q., Chang, F.M., Collier, M.E., Samenuk, G.M., Arris, D.A., Locke, E.E., Bach, S.B.H., Tobon, A., Ruparel, S.B., Hargreaves, K.M., 2021. Elevated dietary omega-6 polyunsaturated fatty acids induce reversible peripheral nerve dysfunction that exacerbates comorbid pain conditions. *Nat Metab* 3, 762–773.
- Brady, C.M., Apostolidis, A.N., Harper, M., Yiangou, Y., Beckett, A., Jacques, T.S., Freeman, A., Scaravilli, F., Fowler, C.J., Anand, P., 2004. Parallel changes in bladder suburothelial vanilloid receptor TRPV1 and pan-neuronal marker PGP9.5 immunoreactivity in patients with neurogenic detrusor overactivity after intravesical reseriferatoxin treatment. *BJU Int* 93, 770–776.
- Chaplin, J.M., Morton, R.P., 1999. A prospective, longitudinal study of pain in head and neck cancer patients. *Head Neck* 21 (6), 531–537.
- Chodroff, L., Bendele, M., Valenzuela, V., Henry, M., Ruparel, S., 2016. EXPRESS: BDNF Signaling Contributes to Oral Cancer Pain in a Preclinical Orthotopic Rodent Model. *Mol Pain* 12.
- Chou, H.C., Chien, C.L., Lu, K.S., 2001. The distribution of PGP9.5, BDNF and NGF in the vallate papilla of adult and developing mice. *Anat Embryol (Berl)* 204, 161–169.
- Cuffari, L., Tesseroli de Siqueira, J.T., Nemer, K., Rapaport, A., 2006. Pain complaint as the first symptom of oral cancer: a descriptive study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 102, 56–61.
- Dale, E.A., Fields, D.P., Devinney, M.J., Mitchell, G.S., 2017. Phrenic motor neuron TrkB expression is necessary for acute intermittent hypoxia-induced phrenic long-term facilitation. *Exp Neurol* 287, 130–216.
- Ducreux, Aude Lafoux; Sabine Lotteau; Corinne Huchet; Sylvie. 2020. 'The contractile Phenotype of Skeletal Muscle in TRPV1 Knockout Mice is Gender-Specific and Exercise-Dependent', *Life*, 10: 233.
- Epstein, J.B., Stewart, K.H., 1993. Radiation therapy and pain in patients with head and neck cancer. *Eur J Cancer B Oral Oncol* 29B, 191–199.
- Fan, W., Zhu, X., He, Y., Li, H., Gu, W., Huang, F., He, H., 2018. Peripheral sympathetic mechanisms in orofacial pain. *J Pain Res* 11, 2425–2431.
- Fitzgerald, M.J., Sachithanandan, S.R., 1979. The structure and source of lingual proprioceptors in the monkey. *J Anat* 128, 523–552.
- Galosi, E., La Cesa, S., Di Stefano, G., Karlsson, P., Fasolino, A., Leone, C., Biasiotta, A., Cruccu, G., Truini, A., 2018. 'A pain in the skin. Regenerating nerve sprouts are distinctly associated with ongoing burning pain in patients with diabetes', *Eur J Pain* 22, 1727–1734.
- Ghurye, S., McMillan, R., 2017. Orofacial pain - an update on diagnosis and management. *Br Dent J* 223, 639–647.
- Gilkey, S.J., Plaza-Villegas, F., 2017. Evaluation and management of orofacial pain. *JAAPA* 30, 16–22.
- Grayson, M., Furr, A., Ruparel, S., 2019. Depiction of Oral Tumor-Induced Trigeminal Afferent Responses Using Single-Fiber Electrophysiology. *Sci Rep* 9, 4574.
- Grayson, M., Arris, D., Wu, P., Merlo, J., Ibrahim, T., Fang-Mei, C., Valenzuela, V., Ganatra, S., Ruparel, S., 2022. Oral squamous cell carcinoma-released brain-derived neurotrophic factor contributes to oral cancer pain by peripheral tropomyosin receptor kinase B activation. *Pain* 163 (3), 496–507.
- Grinspan, D., Fernandez Blanco, G., Allevalo, M.A., Stengel, F.M., 1995. Burning mouth syndrome. *Int J Dermatol* 34, 483–487.
- Hockley, J.R.F., Taylor, T.S., Callejo, G., Willbrey, A.L., Gutteridge, A., Bach, K., Winchester, W.J., Bulmer, D.C., McMurray, G., Smith, E.S.J., 2019. Single-cell RNAseq reveals seven classes of colonic sensory neuron. *Gut* 68 (4), 633–644.
- Horan, N.L., McIlvried, L.A., Atherton, M.A., Yuan, M.M., Dolan, J.C., Scheff, N.N., 2022. The impact of tumor immunogenicity on cancer pain phenotype using syngeneic oral cancer mouse models. *Front Pain Res (Lausanne)* 3, 991725.
- Jaaskelainen, S.K., Woda, A., 2017. Burning mouth syndrome. *Cephalalgia* 37, 627–647.
- Kasemeier-Kulesa, J.C., Morrison, J.A., Lefcort, F., Kulesa, P.M., 2015. TrkB/BDNF signalling patterns the sympathetic nervous system. *Nat Commun* 6, 8281.
- Keefe, F.J., Manuel, G., Brantley, A., Crisson, J., 1986. Pain in the head and neck cancer patient: changes over treatment. *Head Neck Surg* 8, 169–176.
- Kim, S.N., Jung, Y.S., Kwon, H.J., Seong, J.K., Granneman, J.G., Lee, Y.H., 2016. Sex differences in sympathetic innervation and browning of white adipose tissue of mice. *Biol Sex Differ* 7, 67.
- Klein, A., Solinski, H.J., Malewicz, N.M., Jeong, H.F., Sypek, E.I., Shimada, S.G., Hartke, T.V., Wooten, M., Wu, G., Dong, X., Hoon, M.A., LaMotte, R.H., Ringkamp, M., 2021. Pruriception and neuronal coding in nociceptor subtypes in human and nonhuman primates. *Elife* 10.
- Lam, D.K., Schmidt, B.L., 2011. Orofacial pain onset predicts transition to head and neck cancer. *Pain* 152, 1206–1209.
- Li, L., Rutlin, M., Abaira, V.E., Cassidy, C., Kus, L., Gong, S., Jankowski, M.P., Luo, W., Heintz, N., Koerber, H.R., Woodbury, C.J., Ginty, D.D., 2011. The functional organization of cutaneous low-threshold mechanosensory neurons. *Cell* 147, 1615–1627.
- K.A. Lindquist S. Belugin A.H. Hovhannisyann T.M. Corey A. Salmon A.N. Akopian 'Identification of Trigeminal Sensory Neuronal Types Innervating Masseter Muscle', *eNeuro* 2021 8.
- LoCoco, P. M., A. L. Risinger, H. R. Smith, T. S. Chavera, K. A. Berg, and W. P. Clarke. 2017. 'Pharmacological augmentation of nicotinamide phosphoribosyltransferase (NAMPT) protects against paclitaxel-induced peripheral neuropathy', *Elife*, 6.
- X. Luo O. Chen Z. Wang S. Bang J. Ji S.H. Lee Y. Huh K. Furutani Q. He X. Tao M.-C. Ko A. Bortsov C.R. Donnelly Y. Chen A. Nacklek T. Berta R.-R. Ji IL-23/IL-17A/TRPV1 axis produces mechanical pain via macrophage-sensory neuron crosstalk in female mice *Neuron* 109 17 2021 2691 2706.e5 e5.
- Luo, P., Zhang, J., Yang, R., Pendlebury, W., 2006. Neuronal circuitry and synaptic organization of trigeminal proprioceptive afferents mediating tongue movement and jaw-tongue coordination via hypoglossal premotor neurons. *Eur J Neurosci* 23, 3269–3283.
- Marable, D.R., Bowers, L.M., Stout, T.L., Stewart, C.M., Berg, K.M., Sankar, V., DeRossi, S.S., Thoppay, J.R., Brennan, M.T., 2016. Oral candidiasis following steroid therapy for oral lichen planus. *Oral Dis* 22, 140–147.
- Maria, O.M., Syme, A., Eliopoulos, N., Muanza, T., 2016. Single-Dose Radiation-Induced Oral Mucositis Mouse Model. *Front Oncol* 6, 154.
- McKenzie, I.A., Biernaskie, J., Toma, J.G., Midha, R., Miller, F.D., 2006. Skin-derived precursors generate myelinating Schwann cells for the injured and demyelinated nervous system. *J Neurosci* 26, 6651–6660.
- Medici, T., Shortland, P.J., 2015. Effects of peripheral nerve injury on parvalbumin expression in adult rat dorsal root ganglion neurons. *BMC Neurosci* 16, 93.
- Menni, S., Boccardi, D., Crosti, C., 2004. Painful geographic tongue (benign migratory glossitis) in a child. *J Eur Acad Dermatol Venerol* 18, 737–738.
- Nguyen, M.Q., Wu, Y., Bonilla, L.S., von Buchholtz, L.J., Ryba, N.J.P., 2017. Diversity amongst trigeminal neurons revealed by high throughput single cell sequencing. *PLoS One* 12, e0185543.
- Nguyen, M.Q., von Buchholtz, L.J., Reker, A.N., Ryba, N.J., Davidson, S., 2021. Single-nucleus transcriptomic analysis of human dorsal root ganglion neurons. *Elife* 10.
- North, R.Y., Li, Y., Ray, P., Rhines, L.D., Tatsu, C.E., Rao, G., Johansson, C.A., Zhang, H., Kim, Y.H., Zhang, B., Dussor, G., Kim, T.H., Price, T.J., Dougherty, P.M., 2019. Electrophysiological and transcriptomic correlates of neuropathic pain in human dorsal root ganglion neurons. *Brain* 142, 1215–1226.
- Paterson, E.A., Turner, P.V., 2022. Challenges with Assessing and Treating Pain in Research Primates: A Focused Survey and Literature Review. *Animals* 12 (17), 2304.
- Patil, M.J., Hovhannisyann, A.H., Akopian, A.N., McKemy, D.D., 2018. Characteristics of sensory neuronal groups in CGRP-cre-ER reporter mice: Comparison to Nav1.8-cre, TRPV1-cre and TRPV1-GFP mouse lines. *PLoS One* 13 (6).
- Payrits, M., Saghy, E., Cseko, K., Pohoczky, K., Bolcskei, K., Ernsts, D., Barabas, K., Szolcsanyi, J., Abraham, I.M., Helyes, Z., Szoke, E., 2017. Estradiol Sensitizes the Transient Receptor Potential Vanilloid 1 Receptor in Pain Responses. *Endocrinology* 158, 3249–3258.
- Pemberton, K., Mersman, B., Xu, F., 2018. Using ImageJ to Assess Neurite Outgrowth in Mammalian Cell Cultures: Research Data Quantification Exercises in Undergraduate Neuroscience Lab. *J Undergrad Neurosci Educ* 16, A186–A194.
- Ravenelle, R., Neugebauer, N.M., Niedzielak, T., Donaldson, S.T., 2014. Sex differences in diazepam effects and parvalbumin-positive GABA neurons in trait anxiety Long Evans rats. *Behav Brain Res* 270, 68–74.
- Ruparel, S., Bendele, M., Wallace, A., Green, D., 2015. Released lipids regulate transient receptor potential channel (TRP)-dependent oral cancer pain. *Mol Pain* 11, 30.
- Saxena, A., Gnanasekaran, N., Andley, M., 1995. An epidemiological study of prevalence of pain in head & neck cancers. *Indian J Med Res* 102, 28–33.

- Schaser, A.J., Stang, K., Connor, N.P., Behan, M., 2012. The effect of age and tongue exercise on BDNF and TrkB in the hypoglossal nucleus of rats. *Behav Brain Res* 226, 235–241.
- Scheff, N.N., Bhattacharya, A., Dowse, E., Dang, R.X., Dolan, J.C., Wang, S., Kim, H., Albertson, D.G., Schmidt, B.L., 2018. Neutrophil-Mediated Endogenous Analgesia Contributes to Sex Differences in Oral Cancer Pain. *Front Integr Neurosci* 12, 52.
- Scheff, N.N., Alemu, R.G., Klares 3rd, R., Wall, I.M., Yang, S.C., Dolan, J.C., Schmidt, B. L., 2019. Granulocyte-Colony Stimulating Factor-Induced Neutrophil Recruitment Provides Opioid-Mediated Endogenous Anti-nociception in Female Mice With Oral Squamous Cell Carcinoma. *Front Mol Neurosci* 12, 217.
- Scheff, N.N., Wall, I.M., Nicholson, S., Williams, H., Chen, E., Tu, N.H., Dolan, J.C., Liu, C.Z., Janal, M.N., Bunnett, N.W., Schmidt, B.L., 2022. Oral cancer induced TRPV1 sensitization is mediated by PAR2 signaling in primary afferent neurons innervating the cancer microenvironment. *Sci Rep* 12, 4121.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9, 676–682.
- Schober, A., Wolf, N., Huber, K., Hertel, R., Krieglstein, K., Minichiello, L., Kahane, N., Widenfalk, J., Kalchauer, C., Olson, L., Klein, R., Lewin, G.R., Unsicker, K., 1998. TrkB and neurotrophin-4 are important for development and maintenance of sympathetic preganglionic neurons innervating the adrenal medulla. *J Neurosci* 18, 7272–7284.
- Stucky, N.L., Gregory, E., Winter, M.K., He, Y.Y., Hamilton, E.S., McCarron, K.E., Berman, N.E., 2011. Sex differences in behavior and expression of CGRP-related genes in a rodent model of chronic migraine. *Headache* 51, 674–692.
- S.D. Tardif K.G. Mansfield R. Ratnam C.N. Ross T.E. Ziegler The marmoset as a model of aging and age-related diseases *ILAR J* 52 2011 54 65.
- Tavares-Ferreira, D., S. Shiers, P. R. Ray, A. Wangzhou, V. Jeevakumar, I. Sankaranarayanan, A. M. Cervantes, J. C. Reese, A. Chamesian, B. A. Copits, P. M. Dougherty, R. W. th Gereau, M. D. Burton, G. Dussor, and T. J. Price. 2022. 'Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors', *Sci Transl Med*, 14: eabj8186.
- Usoskin, D., Furlan, A., Islam, S., Abdo, H., Lonnerberg, P., Lou, D., Hjerling-Leffler, J., Haeggstrom, J., Kharchenko, O., Kharchenko, P.V., Linnarsson, S., Ernfors, P., 2015. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* 18, 145–153.
- Vardigan, J.D., Houghton, A.K., Lange, H.S., Adarayan, E.D., Pall, P.S., Ballard, J.E., Henze, D.A., Uslaner, J.M., 2018. Pharmacological validation of a novel nonhuman primate measure of thermal responsivity with utility for predicting analgesic effects. *J Pain Res* 11, 735–741.
- Vierboom, M.P., Breedveld, E., Kondova, I., B., A., t, Hart., 2010. Collagen-induced arthritis in common marmosets: a new nonhuman primate model for chronic arthritis. *Arthritis Res Ther* 12, R200.
- Villa, A., Sonis, S.T., 2015. Mucositis: pathobiology and management. *Curr Opin Oncol* 27, 159–164.
- Wu, P., Arris, D., Grayson, M., Hung, C.N., Ruparel, S., 2018. Characterization of sensory neuronal subtypes innervating mouse tongue. *PLoS One* 13, e0207069.
- Yang, C., Tang, H., Wang, L., Peng, R., Bai, F., Shan, Y., Yu, Z., Zhou, P., Cong, Y., 2018. Dimethyl Sulfoxide Prevents Radiation-Induced Oral Mucositis Through Facilitating DNA Double-Strand Break Repair in Epithelial Stem Cells. *Int J Radiat Oncol Biol Phys* 102, 1577–1589.
- Yang, Z., Wang, K.K.W., 2015. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends Neurosci* 38 (6), 364–374.
- Yang, L., Xu, M., Bhuiyan, S.A., Li, J., Zhao, J., Cohrs, R.J., Susterich, J.T., Signorelli, S., Green, U., Stone, J.R., Levy, D., Lennerz, J.K., Renthal, W., 2022. Human and mouse trigeminal ganglia cell atlas implicates multiple cell types in migraine. *Neuron* 110 (1806–21), e8.
- Zhai, J., Zhou, W., Li, J., Hayworth, C.R., Zhang, L., Misawa, H., Klein, R., Scherer, S.S., Balice-Gordon, R.J., Kalb, R.G., 2011. The in vivo contribution of motor neuron TrkB receptors to mutant SOD1 motor neuron disease. *Hum Mol Genet* 20, 4116–4131.