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Chronic Oral Capsaicin Exposure During Development Leads to Adult Rats with Reduced Taste Bud Volumes

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Abstract

Introduction—Cross-sensory interaction between gustatory and trigeminal nerves occurs in the anterior tongue. Surgical manipulations have demonstrated that the strength of this relationship varies across development. Capsaicin is a neurotoxin that affects fibers of the somatosensory lingual nerve surrounding taste buds, but not fibers of the gustatory chorda tympani nerve which synapse with taste receptor cells. Since capsaicin is commonly consumed by many species, including humans, experimental use of this neurotoxin provides a naturalistic perturbation of the lingual trigeminal system. Neonatal or adults rats consumed oral capsaicin for 40 days and we examined the cross-sensory effect on the morphology of taste buds across development.

Methods—Rats received moderate doses of oral capsaicin, with chronic treatments occurring either before or after taste system maturation. Tongue morphology was examined either 2 or 50 days after treatment cessation. Edema, which has been previously suggested as a cause of changes in capsaicin-related gustatory function, was also assessed.

Results—Reductions in taste bud volume occurred 50 days, but not 2 days post-treatment for rats treated as neonates. Adult rats at either time post-treatment were unaffected. Edema was not found to occur with the 5 ppm concentration of capsaicin we used.

Conclusions—Results further elucidate the cooperative relationship between these discrete sensory systems and highlight the developmentally mediated aspect of this interaction.

Implications—Chronic exposure to even moderate levels of noxious stimuli during development has the ability to impact the orosensory environment, and these changes may not be evident until long after exposure has ceased.

Keywords

Plasticity; Trigeminal; Lingual; Chorda Tympani; Cross-sensory Interaction

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Ethical Approval: All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of Interest: Jacquelyn M. Omelian, Kaeli K. Samson and Suzanne I. Sollars declare that they have no conflict of interest.

Introduction

Capsaicin, the chemical that gives chili peppers their piquancy, is found in pungent members of the pepper family *Capsicum*, and is regularly consumed by humans and animals around the world (Vriens et al., 2009). To say that a food “tastes” spicy is technically a misnomer; in mammals capsaicin binds to vanilloid receptor 1 (TRPV1) found in somatosensory, not gustatory, neurons (Szallasi and Blumberg, 1999). Thus, the burning sensation of capsaicin is a sensory illusion; TRPV1 channels are normally responsible for temperature detection and activation by capsaicin is subsequently interpreted as heat (Lundbaek et al., 2005). The physiological and behavioral effects of capsaicin exposure have been studied across a wide spectrum of clinical and experimental contexts including nociception, metabolism, olfaction, and gustation (e.g., Silver et al., 1985; Silver et al., 1991; Prescott 1999; Ludy et al., 2012; Byrnes and Hayes, 2013; Boucher et al., 2014).

When consumed orally, capsaicin acts on tissue of the tongue innervated by the somatosensory lingual nerve. Of particular interest to the present study are the fungiform papillae, lingual-innervated epithelial structures located on the anterior two-thirds of the tongue. An average human fungiform papilla contains between two and five taste buds, though there is considerable variation in taste bud frequency and density between individuals (Arvidson, 1979; Miller, 1986; Miller and Reedy, 1990). In rats, each fungiform papilla houses a single taste bud which once mature contains approximately 50 taste receptor cells (Hendricks et al., 2004). Tastants access the receptor cells via a small pore at the apex of each papilla, and gustatory information is transmitted to the brainstem via the chorda tympani (CT) branch of the facial nerve. Approximately 75% of innervating nerve fibers within fungiform papillae are lingual/trigeminal in origin, with the remaining 25% of fibers consisting of CT axons which are confined to the taste buds (see Figure 1; Montavon et al., 1996). Capsaicin has been shown to damage substance P containing fibers, such as those in the lingual nerve (Nagy et al., 1982). However, the CT is not damaged by capsaicin (Nagy et al., 1982; Hiura et al., 1990).

It is well established that the CT provides critical support to the taste bud; innervation is necessary for normal taste bud formation and maintenance (Whitehead et al., 1987; Sollars, 2005; Lopez and Krimm, 2006). Following nerve transection (CTX) in rats, all animals exhibit a reduction in taste bud number and volumes, regardless of age at surgery (Härd af Segerstad et al., 1989; Sollars, 2005). However, CTX at neonatal ages leads to permanent reductions in taste bud size and frequency while the same surgery in adulthood is followed by nerve regeneration and functional recovery within several weeks (Hendricks et al., 2004). Adult animals also experience fewer morphological changes than neonates even when regeneration of the chorda tympani nerve is prevented (Härd af Segerstad et al., 1989). Interestingly, aged animals that undergo CTX near the end of the lifespan also display less regeneration of taste buds, as compared to adult animals (He et al., 2012). This combination of poorer outcomes associated with surgery in very young and very old animals suggests a defined period during adulthood in which the taste system is most robust against injury. It is notable that changes in taste buds following CTX occur concurrently with morphological changes to the lingual-innervated papillae themselves, despite the fact that the CT does not directly innervate the papilla proper. Loss of the lingual nerve exclusively (i.e., leaving the

CT intact) also produces developmentally differential effects in which younger animals undergo greater reductions in taste bud count and volume, as well as disrupted papillae morphology (Omelian et al., 2016). In total, the outcomes following either gustatory or trigeminal nerve transection suggest that while the CT and lingual nerves are distinctly different in location and function, an integrated, multimodal relationship exists between the taste and trigeminal systems in the anterior tongue.

These findings are further supported by reports of gustatory and trigeminal damage in various clinical populations. Lingual nerve damage during dental surgery commonly leads to reductions in both trigeminal and gustatory sensitivity (Blackburn, 1990; Zungia et al., 1997; Martos-Fernandez et al., 2014). Similarly, a majority of patients with burning mouth syndrome, which is caused by trigeminal neuropathy (Lauria et al., 2005), also report a diminished or dysfunctional sense of taste (Grushka et al., 1986). Conversely, CT damage may occur following ear infection (otitis media; Bartoshuk et al., 2012) or surgery of the middle ear (Just et al., 2007), as the nerve travels along the tympanic membrane en route to the brainstem. Otologic surgical damage to the CT may cause tongue numbness or tingling, and patients report reduced trigeminal sensitivity to capsaicin (Just et al., 2007). This effect is linearly related to the degree of CT damage, with the greatest increase in capsaicin threshold in patients who experience complete CT transection (Just et al., 2007).

Existing surgical and clinical models of trauma illustrate taste system plasticity but the extreme nature of surgical damage prevents this paradigm from accurately representing the typical orosensory environment. The taste system is regularly exposed to chronic assault by noxious stimuli, such as capsaicin, which may thus provide a vehicle for a naturalistic investigation into the trigeminal/taste relationship (Nagy et al., 2004). Since the CT is not affected by capsaicin (Nagy et al., 1982; Hiura et al., 1990), any effects on the taste system resulting from this trigeminal irritant would be via an effect on the lingual nerve, likely as an interruption in its relationship with the CT.

In the current study, rats were given long-term, daily exposure to a moderate dose of oral capsaicin (5 ppm) and the morphological impact to taste and lingual epithelial tissue was assessed. The effects were studied throughout the developmental time course of taste system maturation to further define the parameters of the cross-modal relationship between these systems. Acute oral capsaicin exposure has been shown to interfere with the perception of gustatory stimuli by humans (Simons et al., 2002) and participants have reported taste suppression following exposure to capsaicin concentrations as low as 1 ppm (Cowart, 1987). However, exposure to a high concentration (100 ppm) of capsaicin leads to suppressed central responses to taste stimuli in rats, a finding attributed to localized edema causing restriction of the taste pore (Simons et al., 2003). Since we use a moderate dose of capsaicin, we also examined whether edema occurs at doses such as those used in the present work.

Methods

In order to assess effects of a commonly ingested neurotoxin on orosensory tissue, rats were given daily oral treatments of a capsaicin-containing sucrose solution. Distinct differences in neural response to injury have been found relative to taste system maturity, which occurs

around 40 days of age (P40) when taste buds reach adult size (Shuler et al., 2004). Thus, in the present study, animals were treated as either neonates or adults, relative to the P40 age point. Tissue was examined at either 2 or 50 days post-treatment, in an effort to establish the preliminary time course of capsaicin's effects on the tongue. These time points were chosen to be consistent with previous reports, in which morphological changes were evident as early as 2 days after adult CTX and significant system recovery was evident by 50 days after surgery (Sollars et al., 2002; Sollars, 2005). Figure 2 illustrates the various treatment conditions. All treatments lasted 40 days total.

Subjects

Female Sprague-Dawley rats, from the breeding colony at the University of Nebraska at Omaha were used in this study. Day of birth was designated as postnatal day 0 (P0) and litters were culled to a maximum of 12 pups. Neonatal rats were housed with dams until P25. After weaning and for all adult animals, rats were housed socially with 2–5 littermates in the same treatment condition, to prevent the transmission of capsaicin between animals in the home cage. Animals were housed in Plexiglas cages, with free access to chow (Teklad) and water, and maintained in humidity and temperature controlled rooms on a 12-hour light/dark cycle. All procedures were approved by and conducted in accordance with the guidelines set forth by the local Institutional Animal Care and Use Committee.

Solutions

In rats, initial exposure to capsaicin is aversive and if exposed to solutions higher than 5 ppm initially, animals will reject the solution and refuse to consume it on subsequent trials (unpublished observation). Thus, although it is lower than what naturally occurs in piquant peppers (e.g., jalapeno peppers are approximately 150 ppm; Dong, 2000), this 5 ppm concentration served as both a readily ingested and gusto-affiliative stimulus, when delivered in a 30% sucrose solution.

For the current study, capsaicin powder (95% pure from *Capsicum* sp.; Sigma-Aldrich, St. Louis, MO) was diluted with 2.5% ethanol in distilled water to 5 ppm (16.5 μ M). The use of ethanol as a capsaicin solvent is standard across both animal and human literature (e.g., Silver et al., 1985; Pellicer et al., 1990; McBurney et al., 1997) as capsaicin powder is water insoluble. Because ethanol was required in the capsaicin solution, the sham solution also contained an equivalent concentration of ethanol (2.5%). All treatment solutions were delivered in a 30% (w/v) sucrose/distilled water solution, prepared daily and administered to the animals at room temperature.

Neonatal treatments

Sixteen neonates (8 capsaicin, 8 sham) began daily treatments on P5, and received treatment for 40 days. Animals were removed from nursing mothers, and maintained in a Plexiglas cage on a heating pad. A cotton-tipped applicator was saturated in solution (capsaicin or sham) and rubbed across the surface of the tongue for 3 seconds. This procedure was repeated every 10 minutes for 1 hour (6 times total) and resulted in young animals consuming approximately 1% of their body weight in solution. After the hour, treatments concluded with a brief rinse of distilled water (again via cotton-tipped applicator) to prohibit

capsaicin residue in the oral cavity from irritating the nursing dams. Following treatment, pups were returned to home cages and were observed resuming normal nursing behaviors without delay.

Once rats were weaned (at P25), treatments were administered in hanging cages for 1 hour, and animals were given free access to solution (amount equivalent to 1% of animals' body weight in ml) in calibrated water bottles. Hanging cages were utilized to allow animals to be housed singly and maintained without access to food or water during the treatment period. Neonate treatment and hanging cage procedures ensured equal exposure (capsaicin in mouth) time of one hour between all animals. Animals readily consumed all treatments within the one-hour exposure time.

Adult treatments

Sixteen (8 capsaicin, 8 sham) adult rats began daily treatments at P40 and were also treated for 40 consecutive days. Treatments followed the same protocol as used for weaned neonates.

Tissue Collection and Analysis

In an effort to establish the time course of any morphological changes, half the animals from each treatment condition were sacrificed at 2 days post-treatment and the remaining animals were sacrificed at 50 days post-treatment (resulting in 4 animals in each condition). These time points were chosen to coincide with observed changes and regeneration in surgical studies (Sollars et al., 2002; Omelian et al., 2016).

Animals were given an overdose of ketamine/xylazine and were perfused with a modified KREBS solution, followed by 8% paraformaldehyde. Once tongues were removed, tissue was stored in an 8% paraformaldehyde solution. Taste bud volumes at the tongue tip have been shown to have a higher degree of size variability between buds than elsewhere on the anterior tongue (Krimm and Hill, 1998), thus the anterior-most 2 mm of the tongue tip was removed to allow for consistent volume measurements and to align with previous works (Krimm and Hill, 1998; Hendricks et al., 2004; Sollars et al., 2002; Sollars, 2005; Omelian et al., 2016). Tissue was then frozen and 300 coronal, serial sections (10 μ m, each) were obtained using a cryostat, and sections were mounted on gel-coated slides. Hematoxylin and eosin staining, followed by coverslipping with DPX mounting medium, allowed for visualization of taste buds.

Tissue analysis was conducted using brightfield microscopy, and Neurolucida software (MBF Bioscience) was used to digitally measure taste bud volumes. Briefly, once a taste bud was visualized within a papilla, the circumference of the bud was outlined. This procedure was repeated for each subsequent 10 μ m section, until the entire taste bud was traced (see Figure 3). If any section of a taste bud was obstructed, the entire taste bud was omitted from analysis. After tracing, taste bud volumes were calculated (area multiplied by section thickness; 10 μ m) and summed using NeuroExplorer (MBF Biosciences) software. During taste bud analysis, tissue was also evaluated for the presence of morphological alterations of papillae, such as are common following CTX (Sollars, 2005). All tracings and observations were performed by a single trained individual, blind to the treatment condition.

Edema

An additional component to this study examined the effects of acute capsaicin on edema in tongue epithelium. Consistent with previous work (Simons et al., 2003), adult animals ($N=2$) were given an i.p. overdose of ketamine/xylazine followed by an intracardial injection of Evans Blue dye (0.4 ml/100 g body weight). After dye injection, capsaicin (dissolved in 2.5% ethanol, and delivered in a 30% sucrose solution) was rubbed across the tongue with a cotton-tipped applicator for 5 minutes, and then the animal was perfused with saline. Edema was assessed following exposure to either the 5 ppm capsaicin concentration used in the present study, or after a 100 ppm positive control test. Following capsaicin exposure, tongue tissue was removed and photographed. Edema was qualitatively assessed by the presence (or lack) of visible blue staining in the tongue epithelium and within fungiform papillae.

Data Analysis

Body weights of control and experimental animals were compared with *t-tests* at both the start and conclusion of the 40-day treatment period. This was done to ensure that neither treatment impacted neonatal or adult animals' growth or overall health.

Taste bud size was compared between age-matched capsaicin or sham-treated animals to assess the effects of capsaicin exposure at different developmental ages, and following differing lengths of recovery time. In order to be consistent with previous literature (e.g., Krimm & Hill, 1998; Mistretta et al., 1999; Hendricks et al., 2004; Shuler et al., 2004; Sollars, 2005; Omelian et al., 2016) taste bud volumes were combined within treatment group, each consisting of 4 animals and 134–153 total taste buds per group. One-way ANOVAs with a Tukey post hoc test were run to assess within group variance, and identify any outliers (for ANOVA results, see Table 1). Because significant variance was identified within treatment groups for taste bud volumes, non-parametric Mann-Whitney tests were utilized.

Results

Animal Health

Neonates began treatment on P5 (weighing 11–16 g), while adults began treatment on P40 (weighing 140–186 g). There were no significant differences ($ps > .05$) in weight between experimental and control animals at either the start or end of the experiment (ending weights for neonates sacrificed at P47: 165–192 g; P95: 230–300 g; adults sacrificed at P82: 235–298 g; P130: 258–326 g), suggesting that experimental animals' overall health was not detrimentally affected by any treatment.

Taste bud volume

Capsaicin-related changes in taste bud volumes highlight the susceptibility of the gustatory system to influence by a trigeminal irritant. Animals treated with capsaicin as neonates and sacrificed at 50 days post-treatment had significantly smaller taste bud volumes than age matched sham treated animals, $U=1703$, $p < .001$. Capsaicin treatments did not, however, significantly impact taste bud volumes at two days post-treatment in animals treated as neonates, $U=1874$, $p = .086$. Even when compared with stricter criteria, by collapsing the

data within animals and comparing only the average taste bud volume, capsaicin treated neonates had significantly smaller average taste bud volumes at 50 days post-treatment than those treated as shams ($U = 1$, $p = .043$) while the means of neonatal animals at 2 days post did not significantly differ ($U = 4$, $p = .248$). Thus the damage related to capsaicin appears to be extremely slow acting, with differences not evident until 90 days after initial treatments began and no evidence of change at the earlier time point. Figure 4 shows the distribution of taste bud volumes for neonatal animals.

Taste bud volumes of adult treated animals were not significantly different between treatment groups at either two days post-treatment ($U = 2761$, $p = .463$), or at the 50 day post-treatment time point ($U = 2897$, $p = .813$). This pattern of effects remained consistent when data were collapsed and mean taste bud size for each animal was compared across treatment (adult animals at 2 days post $U = 6$, $p = .564$ or 50 days post $U = 7$, $p = .773$). It appears that the adult taste system is either able to maintain gustatory structures despite the presence of capsaicin or that capsaicin fails to cause damage in these animals. These results corroborate earlier evidence suggesting that the adult taste system is better able to withstand and recover from neural loss than the neonatal system (Sollars, 2005; Omelian et al., 2016). See Figure 5 for the distribution of adult taste bud volumes.

Papillae Morphology

Neither capsaicin nor sham treatments resulted in changes to the observed papillae (e.g., formation of filiform-like papillae; Oakley et al., 1990). Papillae morphology remained unchanged, regardless of treatment age or post-treatment sacrifice date.

Edema

Evans Blue dye was used as a marker for capsaicin-related edema in fungiform papillae. Animals treated with 100 ppm capsaicin, as a positive control, showed extensive blue dye across the entire epithelium and within the papillae. Such staining was not evident in animals treated with acute 5 ppm capsaicin, demonstrating localized edema was not present in these animals and thus not an underlying cause for the changes seen in taste bud volumes (see Figure 6).

Discussion

The current study examined the effects of long-term exposure to moderate doses of orally administered capsaicin on the peripheral taste system across development. Rats received either a 5 ppm capsaicin solution (in 2.5% ethanol) or ethanol-containing sham solution once daily for 40 days, starting at either P5 (for neonates) or P40 (for adults) and tongues were analyzed at either 2 days or 50 days after treatment cessation. Animals that were administered capsaicin before system maturation had significantly reduced taste bud volumes later in life. Once mature, the adult taste system was robust against any influence from capsaicin, regardless of the post-treatment time point. Furthermore, acute edema (as assessed by Evans Blue dye) did not occur at the capsaicin concentration used here, suggesting that the changes in taste bud volume are not related to closure of the taste pore. An earlier investigation, using similar methods, was conducted using 1 ppm (3.3 μ M)

capsaicin, and no significant differences in taste bud volume or papillae morphology were noted between treatment groups in either neonates or adults, at the 2 or 50 days post-treatment time point (unpublished observations).

Lingual nerve damage is the most likely candidate for the effects seen following capsaicin exposure. Systemic capsaicin treatment via i.p. injection leads to a complete retraction of substance P-containing lingual nerve fibers in the fungiform papillae of neonatal rats (Nagy et al., 1982) and topical capsaicin exposure leads to significantly reduced vascular, pilomotor, and sensory nerve innervation in humans (Gibbons et al., 2010). On the tongue, the injurious effects of capsaicin are likely limited to TRPV1-reactive lingual fibers as they pass through the bud en route to epithelial targets in the papillae (Astback et al., 1997; Kido et al., 2003), as the CT is not damaged by capsaicin (Hiura et al., 1990). Similarly, perception of capsaicin appears to be specific to the lingual nerve, since rats that undergo bilateral CTX do not differ in rates of capsaicin consumption at 2 weeks after surgery (Boucher et al., 2014). Lingual nerve transection also results in a reduction of taste bud size and provides evidence of the integrated relationship between the trigeminal and gustatory systems in taste bud maintenance (Omelian et al., 2016). In the present study, reductions in taste bud volume after capsaicin treatment were not accompanied by changes in fungiform papillae morphology, a pattern of effect that mirrors the results of adult lingual nerve cuts (Omelian et al., 2016). Thus, it appears that taste buds are particularly sensitive to disruptions in the cross-sensory relationship of the gustatory and trigeminal systems, while papillae are more resistant, even in instances of complete trigeminal denervation. It should be noted that although the present work strongly suggests that oral capsaicin exposure leads to lingual nerve damage, ethanol is also a trigeminal irritant (Green, 1988) and was used in both the control and capsaicin solutions. A possible interaction between ethanol and capsaicin could also underlie the present findings, though these effects would still implicate the lingual nerve in the observed changes in taste bud size.

The finding that rats treated as neonates had reduced taste bud volumes at the 50-day post-treatment time point, but not at two days post-treatment, suggests that while capsaicin exposure leads to interruption of the developing orosensory environment, the effects occur in a slow-acting manner and do not become apparent until well after the system is mature. Though taste cells regenerate approximately every 10 days (Beidler and Smallman, 1965), taste cell proliferation rates are highest early in development and slow into adulthood (Hendricks et al., 2004). It is possible that capsaicin exposure during development disrupts taste cell proliferation, but due to the normally high rate of new cells produced during the neonatal and early adult ages, this effect is not evident until later in life, when cell proliferation slows naturally. We observed variation in taste bud size within each experimental condition, similar to that typically seen in the taste system of normal adult and developing animals (Krimm and Hill, 1998; Hendricks et al., 2004). It is clearly evident that the taste system is less vulnerable to disruption after system maturation, regardless of injury type. The mechanism for this variation in plasticity across development is unknown, though differences in peripheral immune response are a strong potential candidate (Steen et al, 2010; He et al., 2012).

Lingual nerve damage in humans has been linked to alterations in taste perception as well as decrements in somatosensory sensation (Martos-Fernandez et al., 2014; Shafer et al., 1999; Susarla et al., 2007). Similarly, taste suppression concurrent with acute capsaicin consumption has been found in both humans and rats, particularly the suppression of sweet taste (Prescott and Stevenson, 1995; Simons et al., 2002; Simons et al., 2003). Interestingly, adults who identified as either frequent or infrequent users of capsaicin did not differ in their initial ratings of sweetness perception, nor in the degree of reduction when sweet stimuli were presented concurrently with capsaicin (Lawless et al., 1985; Prescott and Stevenson, 1995), suggesting similar gustatory function regardless of chronic capsaicin consumption. These findings align with the morphological examination in the present work, which found that the adult taste system in rats is resistant to capsaicin related damage. It is not yet known, however, whether humans also undergo changes to gustatory tissue or function following frequent capsaicin exposure at early ages, as was observed here.

It has been more than 100 years since Wilbur L. Scoville quantified the subjective spiciness of peppers, creating the heat index that bears his name. Since then, capsaicin consumption has been studied across a wide array of species and contexts. The present work adds to this rich literature by examining the effects of long-term, moderate dose capsaicin exposure on the developing and mature taste system. Our findings further illustrate the cooperative, multi-modal nature of the gustatory and trigeminal systems in the tongue, and highlight the differences in susceptibility to injury across systems and developmental ages. The exact mechanisms by which the adult and neonatal systems differ will be the focus of future research, and may present interesting implications for informing our understanding of neural susceptibility and plasticity, across the peripheral nervous system.

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Fig. 1.

Illustration of the neural innervation of the rat fungiform papilla and taste bud. The gustatory chorda tympani nerve synapses exclusively with cells within the taste bud, while the somatosensory lingual nerve conveys information from the surrounding papillae epithelium

Fig. 2.

Treatment conditions for evaluating the time course of chronic capsaicin exposure effects across development. Animals, treated as either neonates or adults, were given either capsaicin or sham treatment and tongue tissue was assessed for morphological changes following long-term (40 days) exposure at either 2 or 50 days post-treatment to examine short- and long-term outcomes

Fig. 3.
Photomicrograph of hematoxylin and eosin stained tongue tissue, from a 10 μm coronal section as viewed through Neurolucida (MBF Biosciences). Dotted line represents traced area (taste bud) used to calculate volumes. Scale bar = 50 μm

Fig. 4. Distribution of taste bud volumes, with means, for sham and capsaicin treated neonatal animals, sacrificed 2 or 50 days post-treatment. *Significant differences between mean distribution of taste bud volumes at 50 days post-treatment, $p < .05$

Fig. 5. Distribution of taste bud volumes, with means, for sham and capsaicin treated adult animals, sacrificed 2 or 50 days post-treatment

Fig. 6.

Tongue stained with Evans Blue dye. (A) Tongue surface of an adult animal treated with 100 ppm capsaicin, positive control. Arrow indicates location of (B) enlarged image of a stained fungiform papilla of same animal. (C) Tongue surface of animal treated with 5 ppm capsaicin (treatment concentration). Arrow indicates location of (D) enlarged image of a stained fungiform of same animal. Reduction in amount of dye apparent in C-D suggests a lack of inflammation following 5 ppm capsaicin exposure

Table 1

One-way ANOVA Results Testing Within Group Variance

Treatment Group:	One-Way ANOVA result:
Neonate, Capsaicin, 2d sacrifice	$F(3,69) = 2.46, p = .07$
Neonate, Sham, 2d sacrifice *	$F(3,58) = 3.06, p = .04$
Neonate, Capsaicin, 50d sacrifice	$F(3,64) = 1.91, p = .14$
Neonate, Sham, 50d sacrifice *	$F(3,73) = 3.21, p = .03$
Adult, Capsaicin, 2d sacrifice	$F(3,72) = 2.12, p = .11$
Adults, Sham, 2d sacrifice *	$F(3,74) = 3.74, p = .03$
Adult, Capsaicin, 50d sacrifice	$F(3,75) = 0.99, p = .41$
Adult, Sham, 50 d sacrifice *	$F(3,71) = 3.71, p = .02$

*
 $p < .05$