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EPITHELIAL OVEREXPRESSION OF BDNF AND NT4 PRODUCE DIVERSE GUSTATORY AXON MORPHOLOGIES.

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Introduction

During development, the neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4) are important for establishing and/or maintaining innervation to fungiform taste buds. Previous results from our laboratory demonstrate that altering the pattern of BDNF or NT4 expression in lingual epithelium disrupts normal taste bud innervation. Specifically, when either BDNF (BDNF-OE) or NT4 (NT4-OE) is overexpressed in the basal epithelium of transgenic mice, approximately 69% of fungiform papillae and taste buds were lost. This loss was due to a failure of chorda tympani neurons to innervate taste buds. The current study expands on these results by answering the following questions:

1) Do targeting errors normally occur during peripheral development of the fungiform taste system?
2) Do BDNF-OE mice show the same pattern of altered innervation as NT4-OE mice?
3) When during development is chorda tympani innervation altered by neurotrophin overexpression?
4) Are the targeting effects in BDNF-OE and NT4-OE specific to taste afferents or is somatosensory innervation of fungiform papillae also disrupted?

Results

Gustatory innervation is precise, only a few targeting errors occur during the normal innervation of fungiform papillae.

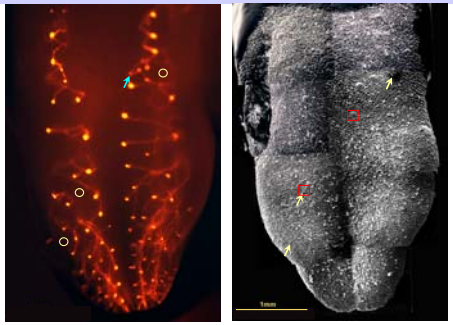

Figure 1. Gustatory innervation patterns are demonstrated by DiI-labeling of the facial nerve (left). Fibers from the chorda tympani branch as they near the tongue surface and innervate specific regions of the epithelium. The locations of fungiform papillae can be seen in a composite SEM micrograph of the same tongue (right), and the number of successfully innervated papillae can be quantified. In general gustatory innervation is precisely targeted to fungiform papillae; however, some targeting errors did occur. While most fungiform papillae are innervated, three papillae on the depicted tongue are not (yellow arrows). In addition, chorda tympani fibers innervate one location on the tongue where no fungiform is present (blue arrow). In three tongues examined with both DiI and SEM, a total of 5 papillae were not innervated and 8 locations without fungiform papillae were innervated.

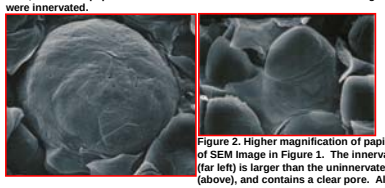

Figure 2. Higher magnification of papillae in red box of SEM Image in Figure 1. The innervated papilla (far left) is larger than the uninnervated papilla (above), and contains a clear pore. All of the uninnervated papillae examined were degenerating and adopting filiform-like morphologies.

Epithelial overexpression of BDNF and NT4 each produce distinct spatial patterns of altered chorda tympani innervation.

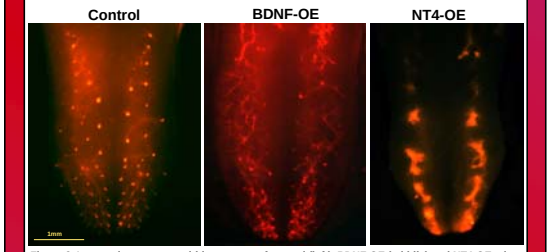

Figure 3. Innervation patterns within tongues of control (left), BDNF-OE (middle) and NT4-OE mice are demonstrated by DiI-labeling of the facial nerve of E18.5 embryos. In BDNF-OE mice, many thin processes are present near the epithelial surface; however, most fail to innervate fungiform papillae. In the NT4-OE mice, branching is reduced and the fibers are more fasciculated than in controls.

Epithelial overexpression of BDNF disrupts branching patterns, resulting in the innervation of non-gustatory epithelium.

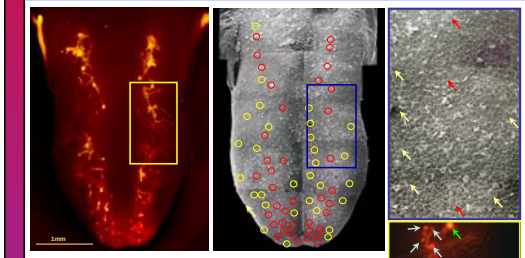

Figure 4. Innervation pattern (left) and fungiform papillae distribution (middle) in the tongue of a BDNF-OE mouse on E18.5. Specific regions of the tongue can be viewed to the right at a higher magnification. While 37 papillae on the dorsal tongue surface receive innervation from the chorda tympani (red arrows and circles), 30 papillae remained uninnervated (yellow circles and arrows). Successfully innervated fungiform papillae are only present along a narrow strip of innervation on each side of the tongue midline. BDNF-OE disrupts chorda tympani branching patterns and causes many regions of non-gustatory epithelium to be innervated (light blue arrows). The green arrows point to locations where innervation successfully reaches fungiform papillae (red arrows).

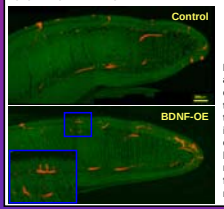

Figure 5. Composite confocal images of a section from a control and BDNF-OE tongue on E18.5. Branches extend toward the epithelia surface from the base of the both tongues. In control mice, specific regions of the epithelium containing fungiform papillae are innervated. In BDNF-OE mice, wider areas of the epithelium are innervated, and many innervated areas lack fungiform papillae. The blue box designates a region where filiform papillae are innervated by the facial nerve and can be viewed at a higher magnification.

Epithelial overexpression of NT4 increases fasciculation and inhibits projections to the epithelial surface.

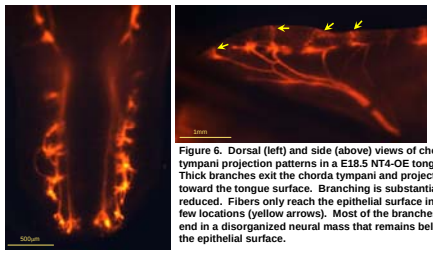

Figure 6. Dorsal (left) and side (above) views of chorda tympani projection patterns in a E18.5 NT4-OE tongue. Thick branches exit the chorda tympani and project toward the tongue surface. Branching is substantially reduced. Fibers only reach the epithelial surface in a few locations (yellow arrows). Most of the branches end in a disorganized neural mass that remains below the epithelial surface.

Neurotrophin overexpression disrupts chorda tympani overexpression patterns by E14.5.

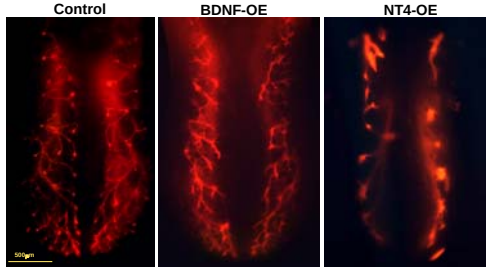

Figure 7. Gustatory innervation patterns within the whole tongue are demonstrated by DiI-labeling of the facial nerve of E14.5 embryos. In control mice, chorda tympani fibers have successfully innervated many fungiform papillae (left). In BDNF-OE mice many fine branches are present that do not appear to innervate fungiform papillae. However, some fungiform papillae near the midline of the dorsal tip and around the intermolar eminence are innervated. Altered patterns of innervation have also already occurred in NT4-OE mice by E14.5 (right). In these mice, branching near the epithelial surface is completely inhibited. Because E14.5 is the first embryonic day in which fungiform papillae are innervated, it is unlikely that chorda tympani fibers initially innervate fungiform papillae and then withdraw in BDNF-OE or NT4-OE mice. Thus, the branching characteristics of chorda tympani fibers are altered during initial tongue innervation.

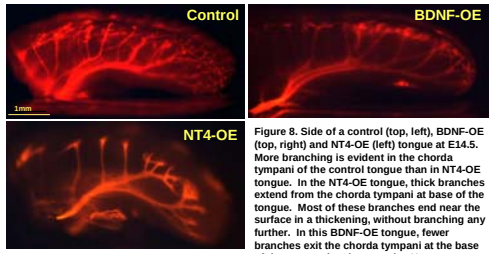

Figure 8. Side of a control (top, left), BDNF-OE (top, right) and NT4-OE (left) tongue at E14.5. More branching is evident in the chorda tympani of the control tongue than in NT4-OE tongue. In the NT4-OE tongue, thick branches extend from the chorda tympani at base of the tongue. Most of these branches end near the surface in a thickening, without branching any further. In this BDNF-OE tongue, fewer branches exit the chorda tympani at the base of the tongue than in controls. However, extensive branching occurs at the epithelial surface.

Most, but not all, fungiform papillae are innervated by trigeminal afferents in BDNF-OE and NT4-OE mice at birth.

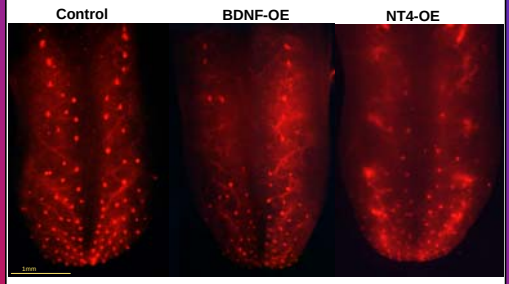

Figure 9. DiI-labeling of the trigeminal demonstrates somatosensory innervation of whole tongues. Most fungiform papillae are innervated by the trigeminal in both BDNF-OE and NT4-OE mice. There is some loss of innervation to the fungiform papillae in the tongue mid-region and around the intermolar eminence. This loss of innervation is greater in NT4-OE tongues than for BDNF-OE tongues and some fasciculation of trigeminal fibers can be seen in the tongues of NT4-OE mice. Overall, the effects of BDNF and NT4 overexpression are much less severe for somatosensory than for gustatory innervation to the tongue.

Conclusions

1) Normal innervation to the tongue is characterized by a stereotypical branching pattern that results in precise targeting of chorda tympani afferents to fungiform papillae. However, a small number of targeting errors do occur during normal development.

2) Epithelial overexpression of BDNF or NT4 each produce distinctive chorda tympani fiber morphologies. NT4 overexpression decreases branching and increases fasciculation which prevents chorda tympani fibers from reaching the epithelial surface. BDNF overexpression encourages branching but disrupts normal branching patterns which misdirects chorda tympani fibers to inappropriate targets. Consistently, in geniculate ganglion explants axonal outgrowth is more fasciculated in response to NT4 stimulation than with BDNF stimulation (Rochlin et al., 2000). Thus, BDNF and NT4 may be important regulators of gustatory axons morphologies during normal development.

3) Chorda tympani innervation is altered by embryonic day 14.5, indicating that neurotrophin overexpression alters innervation patterns as chorda tympani fibers first innervate fungiform papillae.

Acknowledgements

Supported by NIH grants DC04763 and DC05252. We thank Beverly Giammara for help with scanning electron microscopy and the Rolling Stones for providing logo art.