BDNF IS REQUIRED FOR TARGETING OF GUSTATORY FIBERS DURING DEVELOPEMENT

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Introduction

Brain-derived neurotrophic factor (BDNF), normally expressed in the fungiform papillae, is important in gustatory innervation, maintenance of taste buds, and neuronal survival during embryonic development. Previous work from our lab demonstrated that ectopic overexpression of BDNF throughout the tongue epithelium misdirects chorda tympani fibers to innervate non-gustatory filiform papillae. This finding suggests that BDNF is an important target selection, which allows gustatory neurons to recognize and innervate fungiform papillae during development. The purpose of this study is to determine if BDNF is required for gustatory fibers to initially innervate fungiform papillae during embryonic development.

Methods

To determine whether BDNF is necessary for chorda tympani fibers to initially innervate fungiform papillae during embryonic development, innervation to the tongue was examined in wild-type and BDNF null mutant mice (BDNF $^{-/-}$) at embryonic day 14.5 (E14.5), E16.5 and E18.5 of development. Embryos were obtained from overnight mating and the appearance of vaginal plugs the next morning constituted embryonic day E0.5. Embryos were perfused and fixed in 4% paraformaldehyde, then DiI was placed in either of two locations, the geniculate ganglion or in the ear. For geniculate ganglion labeling, embryo brains were lifted out, and DiI crystals applied to the geniculate ganglion. For ear labeling, the outer ear was cut, exposing the inner ear, and DiI crystal placed inside the incision. The embryos were held at 37ºC for 2-12 weeks depending on age. Scanning electron microscopy was used to visualize fungiform papillae on tongue surfaces. Tongue sections were examined with confocal laser scanning microscopy, revealing innervation patterns to non-gustatory (mainly filiform) papillae.

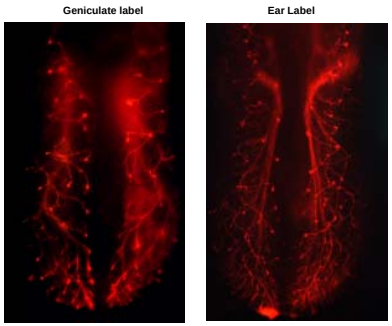

Figure 1. Whole tongues labeled with DiI placed near the geniculate ganglion (above left) or in the inner ear (above right). Both labeled produced the same pattern of chorda tympani innervation in the tongues of E14.5 wild-type mice.

Acknowledgements

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Results at Embryonic day 14.5

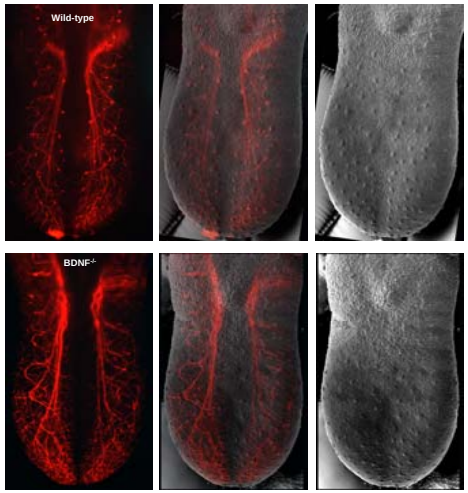


Figure 2. The pattern of Innervated papillae in control tongue (top left) are precise and orderly. SEM Image (top right) showed all the fungiform papillae. The top center image depicts the DiI and SEM merged and it is clear that most fiber bundles were directed to individual fungiform papillae. In BDNF$^{-/-}$ mice (bottom panels), there was a large amount of additional branching and chorda tymapni fibers no longer appear to be directed to specific locations on the tongue surface.

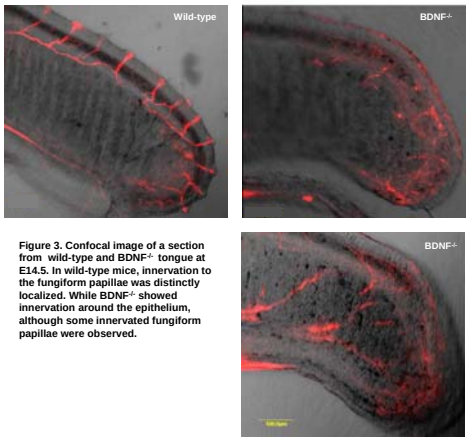


Figure 3. Confocal image of a section from wild-type and BDNF$^{-/-}$ tongue at E14.5. In wild-type mice, innervation to the fungiform papillae was distinctly localized. While BDNF$^{-/-}$ showed innervation around the epithelium, although some innervated fungiform papillae were observed.

Results at Embryonic Day 16.5

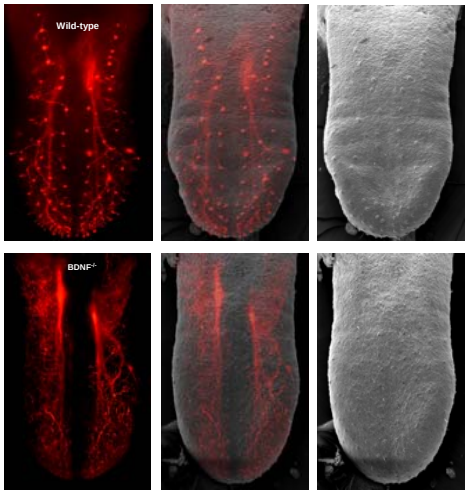


Figure 4. The excess branching of the chorda tympani in BDNF$^{-/-}$ mice continued through E16.5 of development with some innervated fungiform papillae, especially near the tip.

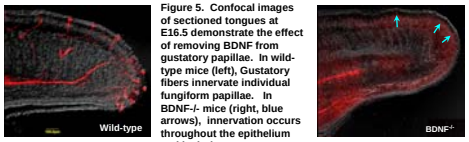


Figure 5. Confocal images of sectioned tongues at E16.5 demonstrate the effect of removing BDNF from gustatory papillae. In wild-type mice (left), Gustatory fibers innervate individual fungiform papillae. In BDNF-/- mice (right, blue arrows), innervation occurs throughout the epithelium and includes some fungiform papillae.

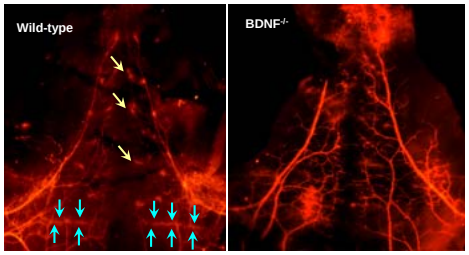
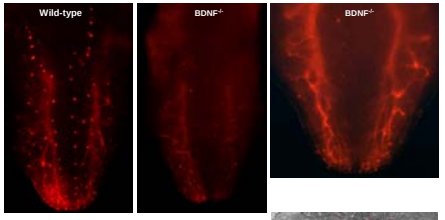


Figure 6. In the soft palate at E16.5 DiI-labeled fibers innervate the geschmacksstreifen (blue arrows) and punctate regions in the posterior palatine field. Although there appears to be substantial innervation to the soft palate in mice lacking BDNF, these specific taste bud containing regions were not innervated.

Results at Embryonic Day 18.5



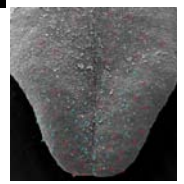


Figure 7. By E18.5 the excess branching was reduced in BDNF$^{-/-}$ mice, and the few fungiform papillae that are innervated can be easily identified. There was also substantial variation in the amount of innervation remaining in the tongue tips of BDNF$^{-/-}$ mice. For example, the tongue tip of the BDNF$^{-/-}$ mouse (top right) was more heavily innervated than the tongue tip of the whole tongue from the BDNF$^{-/-}$ mouse (directly above). SEM analysis of the highly innervated tongue tip (right) revealed that many fungiform papillae still appear to be associated with innervation (red arrows), while many others were clearly not innervated (blue arrows). At E18.5 the caudal tongue and mid-region rarely contained specific innervation in BDNF$^{-/-}$ mice.

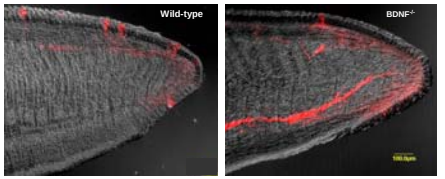


Figure 8. Confocal Image of sections through the tongue tip illustrates the differences in innervation between wild-type and BDNF$^{-/-}$ mice. Innervation to the BDNF$^{-/-}$ tongue (right) shows dispersed branching to the epithelium and fewer innervated fungiform papillae compared to the wild-type tongue (left).

Conclusions

1) In the absence of BDNF, branching in gustatory fibers was initially increased and target selection was disrupted in both the tongue and palate. This finding indicates that BDNF plays an important role in directing chorda tympani fibers to taste buds during development.

2) Even though branching patterns are disrupted in the absence of BDNF, many fungiform papillae were still successfully innervated. This may have occurred because random branching increased the probability that chorda tympani fibers could find their targets by chance. Alternatively, factors other than BDNF may also regulate target selection of gustatory fibers.

3) By E18.5, BDNF$^{-/-}$ mice lost much of the innervation to the tongue. This loss could be due to a retraction of the peripheral fibers that fail to successfully innervate taste buds and/or neuronal death between E16.5 and E18.5.