

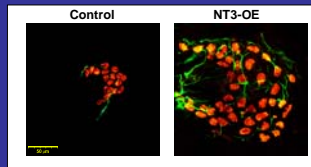
The role of p75 in NT3 enhancement of the touch dome mechanoreceptor

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Introduction

Touch domes are specialized mechanoreceptors containing Merkel cells innervated by slowly adapting type 1 neurons. Their development is dependent on the neurotrophic factor, neurotrophin-3 (NT3). Mice that overexpress NT3 in skin (NT3-OE's) have larger touch domes with both increased innervation and number of Merkel cells (Albers et al., 1996). NT3 overexpression enhances Merkel cell number postnatally and this enhancement is preceded by increased innervation to the touch dome. The pan-neurotrophin receptor, p75, may be important for NT3 regulation of touch dome development. It is known to bind NT3 and is present in both Merkel cells and the neuronal fibers innervating them. Mice that lack p75 have been shown to have a decreased number of Merkel cells in the touch domes. The purpose of this experiment is to determine if p75 is required for NT3 enhancement of Merkel cells and their innervation during postnatal development.



Results

The p75 receptor is required for NT3 enhancement of the number of Merkel cells within a touch dome.

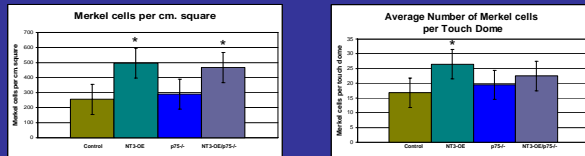


Figure 1. The total number of Merkel cells per cm² and the number of Merkel cells per touch dome were quantified using quinicrine-labeling. * = significantly different from control values.

Absence of p75 enhances innervation to the touch dome Merkel cell complex.

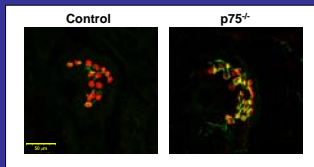


Figure 2. Photomicrographs of touch dome mechanoreceptors. Merkel cells are shown in red while innervation is illustrated in green. Innervation to the touch dome was increased in p75 knockout mice (right) compared with controls (left).

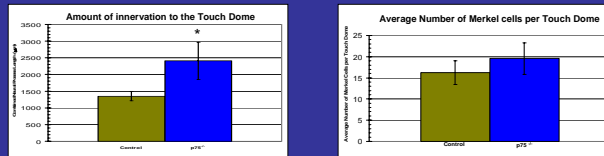


Figure 3. Innervation to the touch dome (left) and mean number of Merkel cells per touch dome (right) for control and p75 knockout mice. p75 knockout mice show an increase in innervation to the touch dome compared to controls ($p < 0.05$). No significant difference exists between the average number of Merkel cells in control and p75^{-/-} animals. * = significantly different from control values.

p75 is required for NT3 enhancement for touch dome innervation

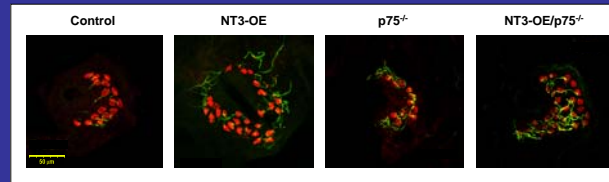


Figure 4. Photomicrographs of touch domes from control, p75 knockout, NT3 overexpressors, and NT3-OE/p75^{-/-} mice. NT3-OE and NT3-OE/p75^{-/-} animals possess similar numbers of Merkel cells. No significant difference exists between NT3-OE/p75^{-/-} and NT3-OE touch domes.

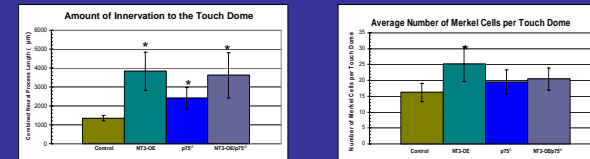


Figure 5. Average amount of innervation to the touch dome (left) and mean number of Merkel cells per touch dome quantified (right) of control, NT3-OE, p75^{-/-}, and NT3-OE/p75^{-/-} animals. The amount of innervation to the touch dome is increased by NT3 overexpression and an absence of p75. The average number of Merkel cells per touch dome is increased only by NT3. There is no difference in the amount of innervation to the touch or the number of Merkel cells within the touch domes between p75^{-/-} and NT3-OE/p75^{-/-}. Therefore, p75 does play a role in NT3 enhancement of the touch dome. The effects of NT3 overexpression and lack of p75 are not additive; this finding indicates that these effects do not occur independently of each other. NT3-OE may enhance innervation to the touch dome by releasing p75 inhibition. * = significantly different from control values.

NT3 overexpression increases touch dome number.

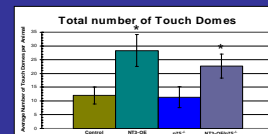


Figure 6. The average number of touch domes per animal. Overexpression of NT3 increases the number of touch domes present. p75 has no effect on the total number of touch domes in an animal.

Conclusions

- 1) Mice lacking the functional p75 receptor have increased innervation to the touch dome mechanoreceptor. Knocking out p75 had no effect on the number of Merkel cells. Thus, p75 normally suppresses innervation to the touch dome.
- 2) NT3 overexpression is less effective at enhancing innervation to the touch dome when a functional p75 receptor is absent.
- 3) The NT3 overexpression effect and the increase in innervation in p75^{-/-} mice are not additive. Thus, these enhancement effects are not independent of one another. NT3 probably increases innervation to the touch dome in part by suppressing the inhibitory effect of p75.

How might the p75 receptor suppress innervation to the touch dome?

- p75 inhibits NT3 binding to trkA in normal development (Mischel et al., 2001). This characteristic of p75 may also explain the increase in innervation to the touch dome; absence of p75 may increase NT3 activation of trkA and cause an increase in innervation to the touch dome.
- p75 is known to activate Rho, a small GTPase that regulates the state of actin polymerization, inhibiting neurite outgrowth. Thus, removal of p75 could reduce Rho activation and increase innervation to the touch dome by locally enhancing neurite outgrowth.
- p75 does not activate Rho when bound to neurotrophic factors. Thus, high levels of NT3 in skin could block p75 inhibition of neurite outgrowth in NT3 overexpressor mice. In this scenario the effects on innervation to the touch dome of NT3-OE and p75^{-/-} mice would not be additive.

Methods

Animals used. Transgenic mice overexpressing NT3 in the epidermis under control of a keratin-14 promoter were used (Albers et al., 1996). p75 knockouts from Jackson Laboratories and NT3-OE's were bred to obtain hybrid NT3-OE/p75^{-/-} mice. The animals were examined at two months of age, birth being defined as postnatal day 0.

Quinicrine labeling. Quinicrine, a fluorescent compound that labels neuroendocrine cells, was injected into mice intraperitoneally (15 mg/kg). After 12–20 hours the animals were deeply anesthetized and killed by cervical dislocation; fresh flank skin was mounted on slides, coverslipped with glycerol and Merkel cells counted at 20x magnification with a Leica microscope with FITC fluorescent optics.

Immunohistochemistry. 50 μ m fresh frozen skin sections fixed in acetone at -20 $^{\circ}$ C were incubated overnight with mouse anti-cytokeratin #20 (DakoCytomation) and rabbit anti-neurofilament M (Chemicon). Secondary antibody labeling with Cy2 and Cy3 was done and the slides coverslipped with DPX mounting solution.

Confocal imaging. Optical sections of each touch dome, spaced 2 mm apart were taken using an Olympus confocal microscope. Red only, green only images were obtained. Each green only image was used to measure the neural process length and this data was summed using NIH imaging software to obtain the combined neural process length, the measure of total innervation to each touch dome.

Acknowledgements

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