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NT3 Expressed in Skin Causes Enhancement of SA1 Sensory Neurons That Leads to Postnatal Enhancement of Merkel Cells

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ABSTRACT

To determine the role of NT3 in the postnatal maturation of Merkel cell (MC) sensory neurite complexes (touch domes), we examined the development of their neural and end-organ components in wild-type and transgenic mice that overexpress NT3 (NT3-OE). Touch domes are sensory complexes of the skin that contain specialized MCs innervated by slowly adapting type 1 (SA1) neurons. Touch domes are dependent on NT3 and, though formed in newborn mice that lack NT3, are severely depleted during postnatal maturation. Mice that overexpress NT3 in the skin have larger touch domes characterized by enhanced neural innervation and MC number. In this study, we asked how this NT3-mediated enhancement occurs, whether through stimulatory effects of NT3 on the SA1 neuron, or the MC, or both. The innervation density and number of MCs associated with each touch dome were measured in wild-type and transgenic animals at postnatal times. In newborn NT3-OE mice, touch dome innervation was enhanced. Surprisingly, however, the number of MCs was lower in newborn NT3-OE animals than in wild-type littermates, and equivalent numbers were not reached until postnatal day 8 (PN8). Not until the PN12 and PN16 time points did MCs increase in NT3-OE mice. To examine the neural dependence of MCs in NT3-OE mice, touch domes were chronically denervated by resecting dorsal cutaneous nerves. Both wild-type and NT3-OE animals showed similar depletion in the number of MCs associated with touch domes. These data indicate that NT3 is not a survival factor for MCs and that the NT3-mediated enhancement of MC number is indirect and neurally dependent. J. Comp. Neurol. 471:352–360, 2004. © 2004 Wiley-Liss, Inc.

Indexing terms: sensory; neurotrophin; development

Sensory innervation of the skin allows an organism to sense temperature, touch, vibration, and painful stimuli. Touch can be conveyed through a unique sensory receptor complex comprising specialized cells of the skin known as Merkel cells (MCs) and slowly adapting type 1 (SA1) mechanosensory neurons. The formation of MC–sensory neuron complexes in the developing skin requires complex trophic interactions between sensory axons and the specialized MCs that they innervate (English et al., 1980; Nurse and Diamond, 1984). How MC–neuron interactions are established and the signaling pathways that underline these neural–target communications are unclear. In mouse back skin, MCs appear at the dermal–epidermal border by embryonic day 15 (E15), with many (61%) already in close juxaposition to developing nerve terminals (Pasche et al., 1990; Saxod, 1996). As maturation proceeds, MCs organize around large tylotrich hair follicles

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and associate with SA1 fibers to form innervated complexes. After MC clusters become innervated, the epithelium above the complex thickens to form the touch dome (Morohunfola et al., 1992). MC and touch dome development is dynamic, with addition of new MCs continuing until at least 2 weeks after birth (Nurse and Diamond, 1984).

MCs are found interdigitated with surrounding keratinocytes of the epidermis. They contain large (50–110 nm) dense-core vesicles and are juxtaposed to axon terminal membranes (Iggo and Muir, 1969). The close spatial relationship among MCs, tylotrich hair and SA1 axons allows mechanical deformation of the skin or deflection of the hair to elicit SA1 activity (Johnson, 2001). The precise role of the MC in mechanosensory transduction is controversial, however, with evidence suggesting that it functions either as a direct transducer (Ikeda et al., 1994; Ogawa, 1996; Senok et al., 1996; Senok and Baumann, 1997) or as a modulator of neural activity (Gottschaldt and Vahle-Hinz, 1981; Mills and Diamond, 1995). Dense-core vesicles of MCs express immunoreactivity for several neuronal compounds (e.g., calcitonin gene-related peptide, substance P, vasoactive intestinal peptide, serotonin, met-enkephalin, somatostatin, peptide histidine isoleucine) that may function as transmitters or modulators of terminal sensitivity (Hartschuh et al., 1979, Hartschuh et al.,1983; English et al., 1992; Leung and Wong, 2000; Tachibana and Nawa, 2002). In addition, MCs express receptors for the metabotropic glutamate receptor 5 (mGluR5) and the ATP receptor P2Y2, indicating responsiveness to these sensory transmitters (Tachibana et al., 2003).

The development and postnatal maturation of MC–neurite complexes provide a unique model system in which to examine how nerve terminals and their target tissues communicate. In the mouse, MCs appear in the embryonic back skin coincident with nerve terminals, suggesting that an intrinsic timing in gene expression and cellular signaling drives early development of each component (Pasche et al., 1990). As maturation proceeds, the question arises of whether the SA1 nerve terminal size dictates development of the mature MC complex or whether addition of new MCs to the touch dome regulates complex maturation. We have approached this issue by examining touch dome development in transgenic mice that overexpress the neurotrophin NT3 in the skin. The formation and maintenance of the neural and MC components of touch dome complexes are highly dependent on NT3 in mice and rats (Airaksinen et al., 1996; Zhou et al., 1998). Mice containing a null mutation in the NT3 gene exhibit a significant postnatal reduction in MC number and SA1 endings. In these animals, total loss of SA1 endings and most MCs occurs by postnatal day 14 (Airaksinen et al., 1996; Krimm et al., 2000). In contrast, mice that overexpress NT3 in epidermal keratinocytes have enlarged touch domes that contain an enhanced number of MCs and neuronal innervation (Albers et al., 1996). This enhanced innervation to Merkel complexes coincides with increased survival of neurons in the dorsal root ganglia (DRG) that express trkC, the receptor tyrosine kinase that preferentially binds NT3 and facilitates trophic signaling (Huang and Reichardt, 2001).

Although it is clear that NT3 plays a fundamental role in the development of touch dome complexes, several scenarios of cause and effect could explain its growth-promoting action. For example, immunolabeling studies indicate that MCs express the p75 receptor and, in the developing whisker follicle cells, the trkC neurotrophin receptor (English et al., 1994; Bergman et al., 2000; Cronk et al., 2002). Thus, NT3 produced by surrounding keratinocytes may directly stimulate production of MCs and cause an increase in MC number independently of SA1 innervation. Evidence also suggests that MCs express several neuropeptides and the neurotrophins nerve growth factor (NGF) and NT3 (Vos et al., 1991; English et al., 1994; Bergman et al., 2000; Leung and Wong, 2000; Szeder et al., 2003). MC production of trophic compounds could stimulate branching of innervating SA1 fibers that, in a feedback manner, would drive MC production. To address how the epidermal level of NT3 modulates touch dome complex development, we first determined the temporal relationship between the postnatal increase in MCs and the density of nerve innervation in wild-type and NT3-OE animals. We also examined the dependence of MCs on neural innervation when in the presence of enhanced NT3. Our analysis indicates that, in NT3-OE mice, enhanced innervation to the touch dome occurs prior to the increase in MC number and that denervation causes a significant reduction in the number of MCs in both wild-type and NT3-OE animals, i.e., excess NT3 in the skin did not increase the survival of denervated MCs. These findings indicate that the effects of skin-derived NT3 are primarily, if not exclusively, on the SA1 nerve terminals and not on their target, the MC.

MATERIALS AND METHODS

Animals

Transgenic mice that overexpress NT3 (NT3-OE) in basal cells of the epidermis under the control of the human keratin-14 promoter/enhancer gene sequences were used (Albers et al., 1996). NT3-OE mice and littermate controls were examined at various postnatal ages as described below. Birth was defined as postnatal day 0 (P0). Genotypes were determined by using transgene specific polymerase chain reactions (PCRs) and/or slot-blot analysis with a ^{32}P-labeled probe to the K14-NT3 transgene. Animals were cared for and used in accordance with guidelines of the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH *Guide for the Care and Use of Laboratory Animals*.

Quinacrine labeling

Touch domes and associated MCs were identified on flank skin by injecting mice intraperitoneally with 15 mg/kg quinacrine dihydrochloride (Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline, pH 7.4 (PBS). After 12–20 hours, mice were deeply anesthetized by Avertin injection and killed by cervical dislocation, and the hair, when necessary, was removed from the flank skin by first shaving and then treating with a depilating agent (Nair). A square piece of back skin (0.5–1 cm^2, depending on the size of the animal) was removed, trimmed of dermal fat and connective tissue, mounted on a glass slide and coverslipped using glycerol. MCs associated with each touch dome were counted at $\times$200 across the entire whole mount by using a Zeiss microscope equipped with fluorescein isothiocyanate (FITC) fluorescent optics.

Immunocytochemistry

Skin samples embedded in OCT and frozen on dry ice were sectioned on a cryostat at 50 μm thickness parallel to the skin surface. Sections were mounted on slides, fixed in –20°C acetone for 10 minutes, air dried, washed in PBS, and then incubated with mouse anticytokeratin 20 (Dako-Cytomation, Carpinteria, CA) and rabbit antineurofilament M (NF M; Chemicon, Temecula, CA) overnight at room temperature. Sections were washed and incubated in goat anti-rabbit Cy2 and goat anti-mouse Cy3 (Jackson, West Grove, PA) for 1–2 hours, washed, and coverslipped with DPX mounting solution (DBH Laboratory Supplies, Poole, England).

Confocal imaging

Optical sections spaced 2 μm apart were collected with a Leica confocal microscope and computer reconstructed. Eight to ten touch domes per animal were analyzed. Green-only, red-only and combined images were collected. By using the green-only image, each neural process within the touch dome was drawn, measured, and then summed to estimate the combined neural process length with the NIH Image software. The number of MCs was counted in the red-only image for the same touch dome. Because these measures were taken separately, the experimenter was blind to the amount of innervation a given touch dome contained when counting MCs and to the number of MCs a particular touch dome contained when measuring innervation.

L4/L5 DRG cell counts and diameters

Cell counts were obtained using methods of Coggeshall et al. (1990). On the day of birth, mice were deeply anesthetized and transcardially perfused with PBS, followed by 4% paraformaldehyde. The L4/L5 DRGs were removed, dehydrated, defatted, and embedded in paraffin. Ganglia were serially sectioned at 5 μm and Nissl stained. Every sixth section was examined at a total magnification of ×400. A drawing tube was used to trace the section outline and demarcate the location of sensory neurons containing a nucleolus. The number of nucleoli counted in each section was multiplied by six, and totals were summed across sections to estimate the total nucleoli number. To compensate for neurons with two or more nucleoli, profiles of randomly selected neurons were reconstructed, and the total number of nucleoli per 100 neurons was determined. This ratio was multiplied by the total number of neurons counted to obtain the total number of neurons per ganglion.

Dorsal cutaneous nerve transection

P17 wild-type and NT3-OE animals were anesthesized with Avertin. Back skin of the animal was shaved, and a midline incision of approximately 0.5 cm in length was made to expose dorsal cutaneous nerves (DCNs). Five or six nerves along one side of the flank (from DRG levels T8–T12) were tied with suture, cut, and then trimmed back to the skin. After 2 weeks of recovery, animals were killed with an overdose of anesthesia, and the skin from both denervated and intact sides was removed. Tissue was frozen on dry ice in OCT, cut on a cryostat, and immunolabeled with antibodies to NF-M and K20 (see above) to detect nerve fibers and MCs in the skin.

Data analysis and photomicrograph production

MC number, combined neural process length, and L4/L5 DRG neuron number were compared between NT3-OE and wild-type mice at each age by using *t*-tests. Analysis of variance (ANOVA) was used to compare MC number and combined neural length across different ages. Pearson product-moment correlations were used to analyze the relationship between number of MCs and combined neural length. The alpha level was set at $P < 0.05$ for statistical comparisons. Photomicrographic files were obtained with a confocal microscope, imported into Adobe Photoshop, and manipulated to enhance contrast and brightness.

RESULTS

NT3 overexpression increases touch dome size by P16

MCs with neurites in close proximity appear in the embryonic mouse back skin at approximately embryonic day 15 (E15; Pasche et al., 1990). Complexes continue to mature postnatally, enlarging in overall size with addition of new MCs (Nurse and Diamond, 1984). Adult mice that express enhanced levels of NT3 in the skin (NT3-OEs) have larger touch domes that contain more MCs and increased innervation (Albers et al., 1996). To define when and how this NT3-mediated enhancement occurs, we first compared the touch domes of wild-type and NT3-OE mice at P16. At P16, touch domes of NT3-OE mice were larger in diameter, had increased innervation density, and contained more MCs (Fig. 1). These findings are consistent with studies of mice that lack NT3, in which a severe depletion of the touch dome–MC complex occurs by P14 (Airaksinen et al., 1996). Therefore, we limited our developmental analysis of the touch dome to animals at P16 and younger.

Effects of elevated NT3 on MC number occur between P8 and P12

To determine when the increase in MC number occurs in NT3-OE mice, touch dome complexes of postnatal animals at various ages were examined. MCs of wild-type and NT3-OE P0 mice were labeled by injection of the fluorescent compound quinacrine and counted the following day. Quinacrine selectively concentrates in neuroendocrine cell types such as the MC and has been used extensively for MC quantification (Nurse and Diamond, 1984; Nurse et al., 1984; Albers et al., 1996; Krimm et al., 2000). We were surprised to find that P1 wild-type mice had more (19%) MCs/touch dome than NT3-OE mice (Fig. 2; $P < 0.05$). Thus, the increased numbers of MCs in NT3-OE mice must accumulate at sometime after P1. We therefore counted the number of quinacrine-labeled cells per touch dome on P8, P12, and P16 and in adult animals for comparison. In this analysis, the number of MCs/touch dome showed a similar increase in both wild-type and NT3-OE mice until P8. However, the number of MCs/touch dome increased in NT3-OE mice at the P12 and P16 time points (Fig. 2; $P < .05$ and $P < .001$, respectively). Thus, enhancement of NT3 expression causes an increase in MC number between P8 and P12.

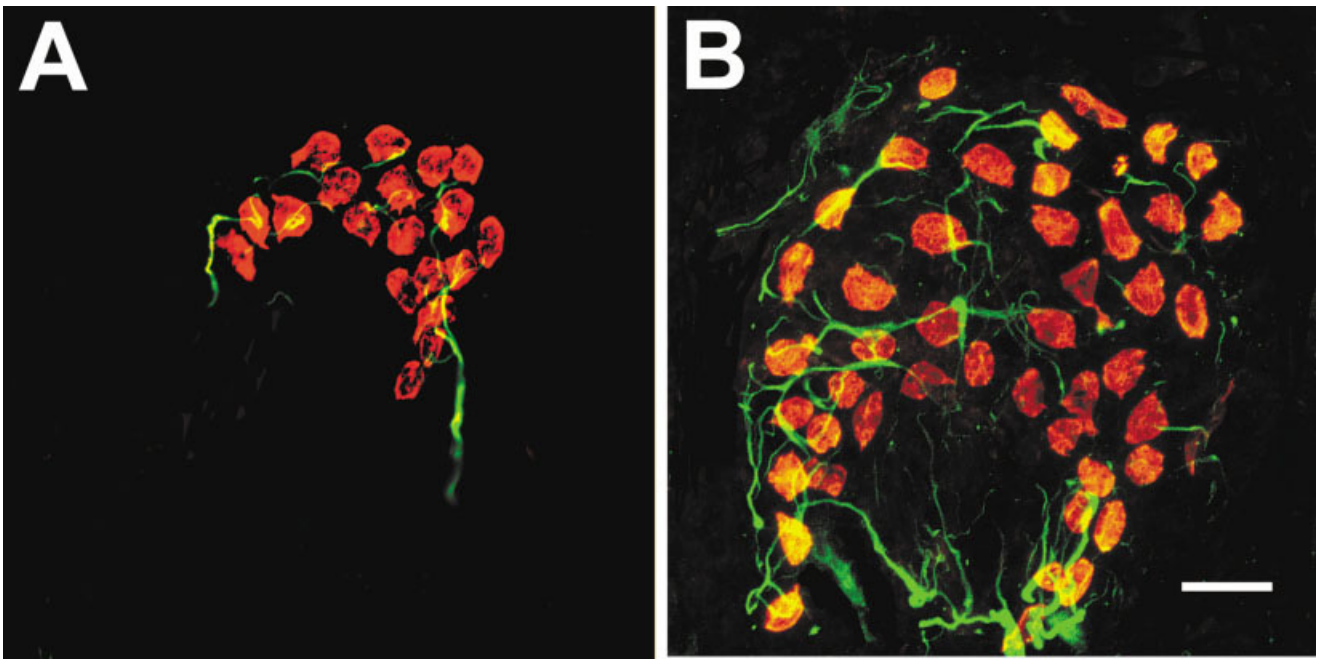

Fig. 1. Confocal micrographs of touch domes at postnatal day 16 in wild-type (**A**) and NT3-OE (**B**) mice. Innervation to the touch dome was detected by immunolabeling with antineurofilament M (green), and Merkel cells were detected with anticytokeratin 20 (red). Touch domes are larger, have more innervation, and have more Merkel cells in P16 NT3-OE skin relative to those in wild-type skin. Scale bar = 20 μm.

NT3 overexpression increases innervation to touch dome complexes by birth

We next asked how enhanced levels of skin-derived NT3 affected maturation of touch dome innervation. That is, does increased innervation density of touch domes in NT3-OE mice precede or follow the increase in MC number? To address this, thick sections (50 μm) of back skin were labeled with anticytokeratin 20 and anti-NF M, and images containing entire touch domes were captured using a confocal microscope. Similar to results obtained using quinacrine labeling (Fig. 2), touch domes of NT3-OE mice had a decreased number of cytokertatin 20-positive MCs at birth ($P < 0.05$); by P8, the numbers of MCs/touch dome were equivalent to those of wild type, and, by P16, their numbers were enhanced (see Fig. 4A; $P < 0.05$). Innervation density was determined in sections from P1 skin (Fig. 3A,B), P8 skin (Fig. 3C,D), and P16 skin (Fig. 1) by measuring the length of NF-M-labeled neural fibers associated with touch dome complexes. The measure of NF-M-labeled processes was defined as the *combined neural process length* for an individual touch dome. Touch domes from NT3-OE mice had a greater combined neural process length at all three postnatal ages examined (Fig. 4B; P1, $P < 0.05$; P8, $P < 0.01$; P16, $P < 0.001$). Therefore, touch domes of NT3-OE mice exhibit enhanced innervation by birth. This developmental increase in innervation is not matched by a similar increase in MC number until more than 8 days later.

During postnatal development of both NT3-OE and wild-type mice, MCs continue to be added to touch dome units (Fig. 4). In contrast to the continual addition of MCs, a postnatal increase in combined neural process length occurs only in NT3-OE touch domes ($P < 0.0005$) and not in wild-type units ($P < 0.17$). Thus, SA1 innervation appears to be completed by birth in wild-type animals but continues postnatally in mice that overexpress NT3 in the skin.

The increased innervation to touch dome complexes postnatally may reflect a continual increase in the numbers of neurons that project to each complex, or an

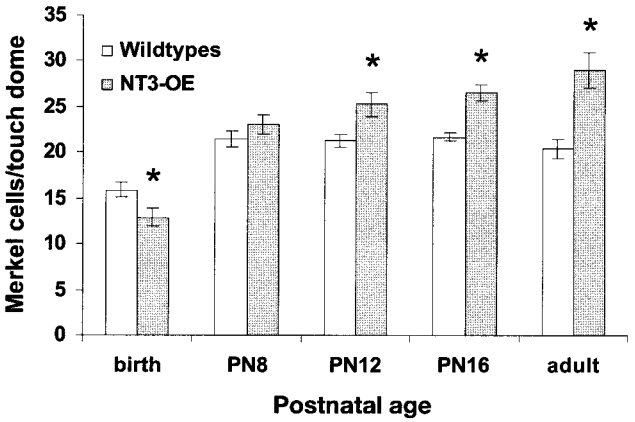

Fig. 2. The number of quinacrine-labeled Merkel cells per touch dome for wild-type (open bars) and NT3-OE (stippled bars) mice across postnatal development (n = 7–8 per group). Note decreased numbers of MCs in NT3-OE touch dome at birth ($P < 0.05$). By postnatal day 12, NT3-OE mice have more Merkel cells per touch dome than wild types ($P < 0.05$). This increase continues at the P16 time point ($P < 0.001$). Asterisks denote statistically significant differences.

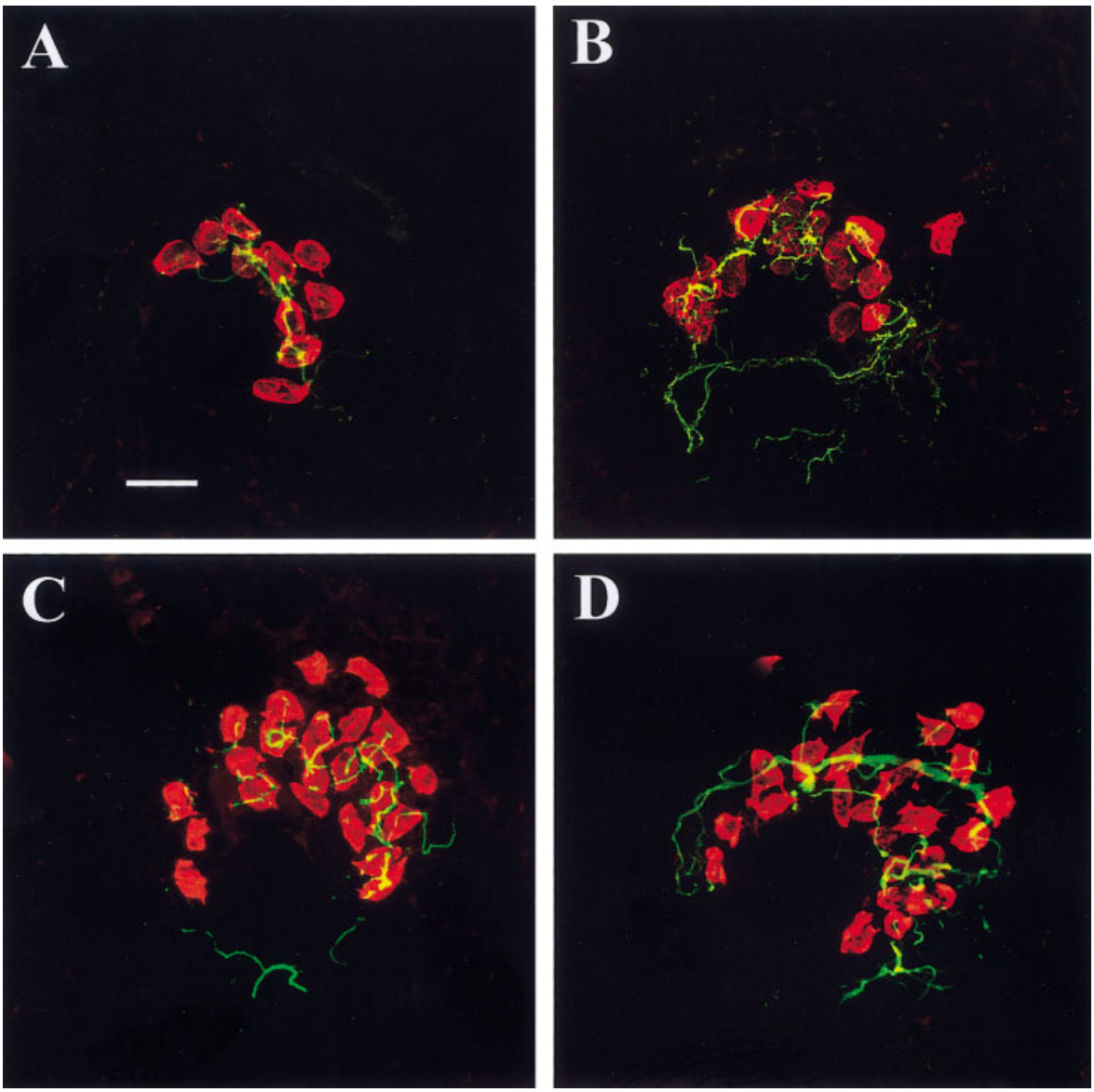

Fig. 3. Confocal micrographs of touch domes from postnatal day 1 (**A,B**) and postnatal day 8 (**C,D**) wild-type (A,C) and NT3-OE (B,D) mice. Merkel cell number is equivalent in wild-type and NT3-OE touch domes at birth and at P8. In contrast, innervation density to NT3-OE touch domes is increased by birth relative to innervation seen in wild-type touch domes. Scale bar = 20 μm.

increase in the amount of nerve branching at the complex (terminal arborization), or both. To address whether more neurons project to developing complexes, the number of sensory neurons in the DRG of postnatal animals was determined. Previous studies of adult NT3-OE mice showed the L4/L5 DRG had approximately 40% more neurons than wild-type DRG (Albers et al., 1996; Krimm et al., 2000). Cell counts of the number of DRG neurons at P0 showed that NT3-OE mice had 37% more neurons than wild type (NT3-OE, 20,176 ± 240; wild type, 14,678 ± 804; $P < 0.02$). This increase in DRG number is consistent with the idea that more neurons innervate the touch domes of NT3-OE mice by P0 and account for the increase in innervation. In that the number of DRG neurons in P0 NT3-OE mice is similar to that measured in the adult ganglia, NT3-OE mice likely have their full complement of additional DRG neurons by birth. The postnatal increase in innervation to the touch dome therefore most likely is due to elaboration of the terminal arborizations of sensory neurons and not the addition of new neurons in the DRG.

The number of MCs/touch dome correlates with increasing innervation

To examine the relationship between innervation density and MC number for individual touch domes, the number of MCs/touch dome was plotted against the

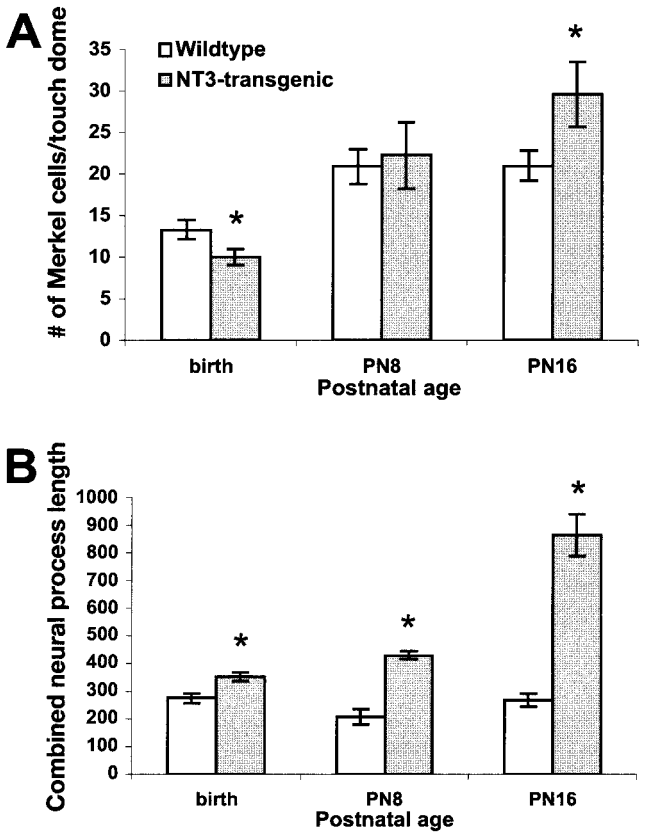

Fig. 4. Quantification of Merkel cell number (**A**) and innervation density (**B**) in touch domes from confocal images of immunocytochemically stained tissues. Similar to the results obtained using quinacrine-labeling, NT3-OE mice had fewer MCs/touch dome at birth compared with wild-type animals ($P < 0.05$), there was no difference in Merkel cell number at P8, and NT3-OE mice have more MCs/touch dome by postnatal day 16 ($P < 0.05$; A). B shows that touch domes of NT3-OE mice have a greater combined neural process length by birth ($P < 0.05$), at P8 ($P < 0.01$), and at P16 ($P < 0.001$) compared with touch domes from wild-type animals. Therefore, during postnatal development, NT3 overexpression increases innervation to touch domes before an increase in Merkel cell number occurs. Asterisks denote statistically significant differences.

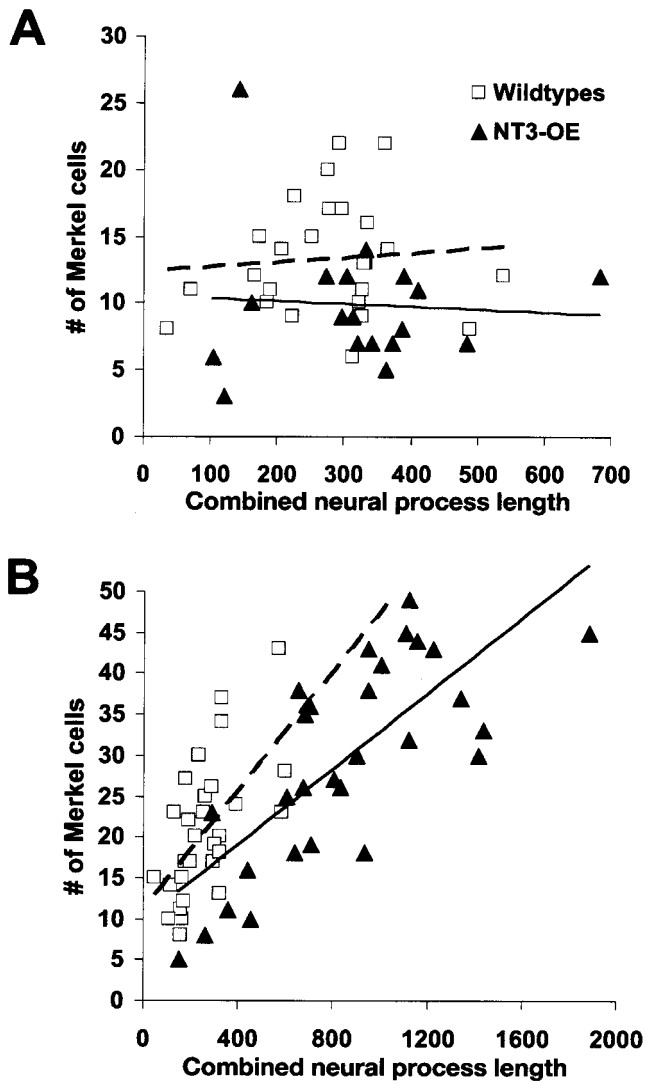

Fig. 5. The number of Merkel cells is plotted as a function of the combined neural process length measured for each touch dome at birth (**A**) and at P16 (**B**) in wild-type (squares) and NT3-OE (triangles) mice. Regression lines plotted for P0 (A) and P16 mice (B) show a positive correlation between the amount of innervation and Merkel cell number for the P16 wild-type ($R = 0.60$; dashed line) and NT3-OE mice ($R = 0.74$; solid line). Thus, by P16, the number of Merkel cells per touch dome can be predicted by the amount of innervation the touch dome receives.

combined neural process length for each touch dome unit. This analysis shows that a correlation between MC number and innervation is not present at birth for either NT3-OE or wild-type mice but rather is established postnatally (Fig. 5A). By P16, the amount of innervation to an individual touch dome predicts the number of MCs it contains for both wild-type and NT3-OE mice (Fig. 5B; $P < 0.01$ for wild type, $P < 0.0001$ for NT3-OEs). This relationship is shifted in NT3-OE mice, with the slope of the regression line reduced in NT3-OE mice relative to the wild-type line (Fig. 5B). Touch domes with large amounts of innervation in NT3-OE mice have fewer MCs than would be predicted from the relationship between innervation and MC number in wild-type mice. This suggests that changes in MC number in touch domes of NT3-OE mice cannot keep pace with the substantial increase in innervation that these touch domes receive during postnatal development.

MCs are dependent on nerve innervation even in the presence of enhanced levels of NT3

That innervation to touch dome complexes was enhanced in newborn (P0) NT3-OE animals, prior to the increase in MC number, indicates that MC number and touch dome size are driven by and dependent on the amount of neural innervation. In NT3-OE transgenics, neural innervation density increases and may stabilize the number of MCs by an as yet unknown mechanism. Indeed, studies in rat have shown that removing innervation to touch dome complexes by cutting and ligating dor-

sal cutaneous nerves causes loss in MC number to 40% of the predenervation average (Nurse et al., 1984). Thus, most but not all MCs are dependent on nerve innervation. Also possible, however, is that the increased level of NT3 in the skin stabilizes MC number in transgenic touch domes and could, for example, protect them from apoptotic stimuli in the developing skin and lead to an increase in MC number. To test whether MCs have greater stability in the presence of increased NT3, we compared their dependence on nerve innervation with that of MCs of wild-type animals. P17 wild-type and transgenic animals, with a full complement of MCs, were used for these studies. Dorsal cutaneous nerves (T8–T12) on the right side were sutured and then cut back from the skin to remove innervation. At 2 weeks postsurgery, MC numbers per touch dome were determined in skin sections from cut and uncut sides. MCs and innervation (if any) were detected by immunolabeling with anti-K20 and anti-NF M, respectively. On the nondenervated side, a normal complement of MCs per touch dome was detected in both wild-type (15.85, n = 4) and NT3-OE (18.93, n = 4) mice, with NT3-OE mice having more MCs ($P < 0.05$), as shown in previous analyses (Albers et al., 1996; Krimm et al., 2000). In contrast, analysis of the denervated skin showed the MC per touch dome ratio to be quite reduced (wild type, 5.48, n = 3; NT3-OE, 6.61, n = 4). Both wild-type and transgenic animals showed a drastic reduction in MC number, with no significant difference between the calculated values of 65% reduction for both lines. Thus, the enhanced level of NT3 produced by the transgenic skin could not maintain MCs in the absence of nerve innervation.

DISCUSSION

In this study, the effect of skin-derived NT3 on the postnatal growth and maturation of the MC neurite complex was examined. The relationship between nerve innervation and end-organ development was assayed using NT3-OE transgenic animals in which enhanced levels of NT3 are expressed in the epidermis. Compared with wild-type littermates, NT3-OE mice exhibit an increase in SA1 innervation density and in the number of associated MCs, which together produce an enlarged touch dome complex. The question we sought to answer was whether NT3-mediated enhancement in touch dome complex size occurred through stimulatory effects of NT3 on the SA1 fiber, or on the MCs, or on both. To test these possibilities, the relative innervation density and MC number were measured in wild-type and transgenic animals at various postnatal times during touch dome maturation. Our findings indicate that the effects of target-derived NT3 on development of touch domes are primarily, if not exclusively, on neural innervation and not on MC development. The following evidence supports this interpretation. First, NT3 overexpression enhanced the density of innervation to the touch dome more than 8 days prior to increasing the MC number in NT3-OE mice. Second, a correlative relationship between MC number and innervation becomes established during postnatal development. This relationship suggests that "communication" between MCs and neural innervation is altered postnatally. Thus, NT3 overexpression, though initiated in transgenic back skin at E12–E13, influences innervation but not MC number until postnatal times. Third, the slope of the relationship between innervation and MC number in NT3-OE mice is lower than that found for wild-type animals. This indicates that, even by P16, the change in MC number cannot keep pace with changing innervation, which must occur first. Fourth, the increased level of NT3 in the skin could not prevent the loss of MCs that normally follows denervation of SA1 input. Neural input was essential for MC survival even in the presence of high levels of NT3; i.e., NT3 by itself does not provide trophic support for MC survival. Therefore, NT3 overexpression in the skin appears initially to enhance innervation to the touch dome site. This increased innervation then stimulates MC development after MCs become dependent on their innervation.

Innervation to touch domes of NT3-OE animals showed a threefold increase relative to wild-type animals by P16, much of which developed postnally. Because the number of DRG neurons did not substantially change in NT3-OE mice postnatally, it seems likely that the enhanced level of NT3 led to branching and elaboration of sensory endings at the touch dome site. This effect is consistent with the finding that NT3 enhances terminal morphology and arborization in cultured DRG neurons (Lentz et al., 1999; Ichinose and Snider, 2000). In addition, NT3 is essential for sensory end-organ formation and elaboration in the whisker pad (Fundin et al., 1997). Taken together, these findings establish NT3 as an in vitro and in vivo regulator of sensory terminal branching characteristics. The receptor and internal signaling mechanisms that mediate these effects are unclear. NT3 binds and can activate trkA, trkB, trkC, and the pan neurotrophin receptor p75 (Davies et al., 1995; Kaplan and Miller, 1997; Farinas et al., 1998; Huang et al., 1999). In culture, trkC activation of the signaling mediator Akt has been shown to change terminal morphology and branching characteristics (Markus et al., 2002), making this a possible means through which branching at the touch dome occurs. However, insofar as NT3 can bind all trk receptors and mice lacking each of these receptors lose some MCs from the palate and back, footpad, and whisker pad skin (Fundin et al., 1997; Kinkelin et al., 1999; Ichikawa et al., 2001; Cronk et al., 2002), receptors other than trkC may mediate the effects of NT3 on touch dome innervation.

The neural dependence exhibited by MCs in the postnatal touch dome complex has been demonstrated by experiments that show loss of MCs in hairy skin following denervation (English et al., 1983). This is a site-dependent response, in that many MCs associated with vibrissae and glabrous footpad skin in rodents are nerve independent, surviving even when denervated (Nurse and Farraway, 1988; Mills et al., 1989). In addition, MCs in the rat oral mucosa appear after birth and after innervation of the epithelium occurs (Tachibana et al., 2000). In the present study, MC neural dependence was demonstrated by measuring the temporal response of developing SA1s and MCs to increased levels of target-derived NT3. The enhancement of MC number associated with NT3-OE touch domes lagged behind the enhancement of the SA1 innervation, suggesting a nerve-driven process. How might neural innervation influence maturation of this sensory complex? One possibility is that the nerve provides a trophic signal that stimulates the production of MCs in the skin and/or blocks death of MCs that might normally occur. A trophic stimulation could occur through release of a factor or via an activity-driven mechanism. In an activity model, the neural activity of the SA1 neurite in the touch dome field

would stimulate production of MCs in the terminal field. SA1 neurons are responsive to glutamate (Morhenn et al., 1994; Genever et al., 1999; Fagan and Cahusac, 2001) and, in the postnatal animal, have mature electrophysiologic profiles and central termination patterns as early as P1 (C.J.W. and H.R. Koerber, unpublished data), making enhanced activity feasible.

A neural-derived trophic substance may also prolong the life span of MCs in the NT3-OE touch dome and block an apoptotic death program. If a trophic substance from the nerve affected MC survival or replication, our results would predict that MC dependence on this unknown nerve-derived factor would develop between P8 and P12. This could account for the more severe loss of MCs observed when the nerve is cut between P1 and P4 (Mills et al., 1989), before nerve dependence develops. A comprehensive study of MC apoptosis and replication in the postnatal touch dome has not as yet been carried out, making the influence of these events on postnatal maturation difficult to judge. Dividing MCs have been detected in embryonic (E12–E14) vibrissal hair follicles in mouse and in the adult human and rabbit skin, although in all cases these were rarely observed (Merot et al., 1987; Vaigot et al., 1987; Moll et al., 1996).

Enhanced numbers of MC could also be generated by stimulation of MC production via cell-to-cell contacts. MCs may arise through division of MCs already populating the skin or from surrounding epidermal stem cells that undergo differentiation into the MC lineage (Moll et al., 1996). If MCs are from skin stem cells, factors that regulate MC differentiation may have altered expression in response to cell-to-cell contacts. Differentiation factors could include components of signaling pathways activated by cell surface contact, which are predicted to be greater in NT3-OEs because of the expanded terminal field. For example, cadherins and integrins are expressed by both sensory neurons and keratinocytes. Activation of signaling pathways downstream of these surface proteins (e.g., via catenin and wnt/frizzled signaling) may occur in either established MCs or skin stem cells in response to neural contact. Enhanced production of MCs would thereby provide additional target sites for the expanded NT3-responsive neurite.

Regulation of sensory end-organ development by afferent input is thought to occur in other sensory systems, such as taste and muscle proprioceptors. In muscle, afferent-derived factors are hypothesized to underlie the induction and differentiation of muscle spindle mechanoreceptors. In a recent study, sensory neuron expression of neuregulin 1 was found to be required for muscle spindle differentiation, as evidenced by intrafusal fiber expression of transcription factors Egr3, Pea3, and Erm (Hippenmeyer et al., 2002). A model was proposed in which neuregulin 1 from the sensory afferent acts through ErbB receptors to elicit transcriptional changes at the site of spindle development and thereby regulate differentiation.

The findings of this study indicate that the development and postnatal maturation of the MC–neurite sensory complex are driven primarily by sensory neuron response to NT3 production in the target, which in turn regulates MC complex size. Thus, in animals that lack NT3, it is likely that the degenerative effects on SA1 nerve fibers caused by NT3 deprivation drive the postnatal loss of MCs. Conversely, enhancement of neuron terminal size in NT3-OE animals is hypothesized to be the driving force for MC enrichment and touch dome growth. Another interpretation of this data may be made, however, in which NT3 overexpression in the skin is actually inhibitory to developing MCs. Inhibition is suggested by the initial reduction in MC number in transgenic touch domes of P1 NT3-OE animals. How this inhibition could occur is purely a matter of speculation without additional data on touch dome formation in embryonic NT3-OE mice. However, if this is the case, then the enhancement of SA1 innervation at the touch dome site may provide a positive (or neutralizing) effect that allows MC enhancement at later postnatal times. Future studies will explore this possibility.

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